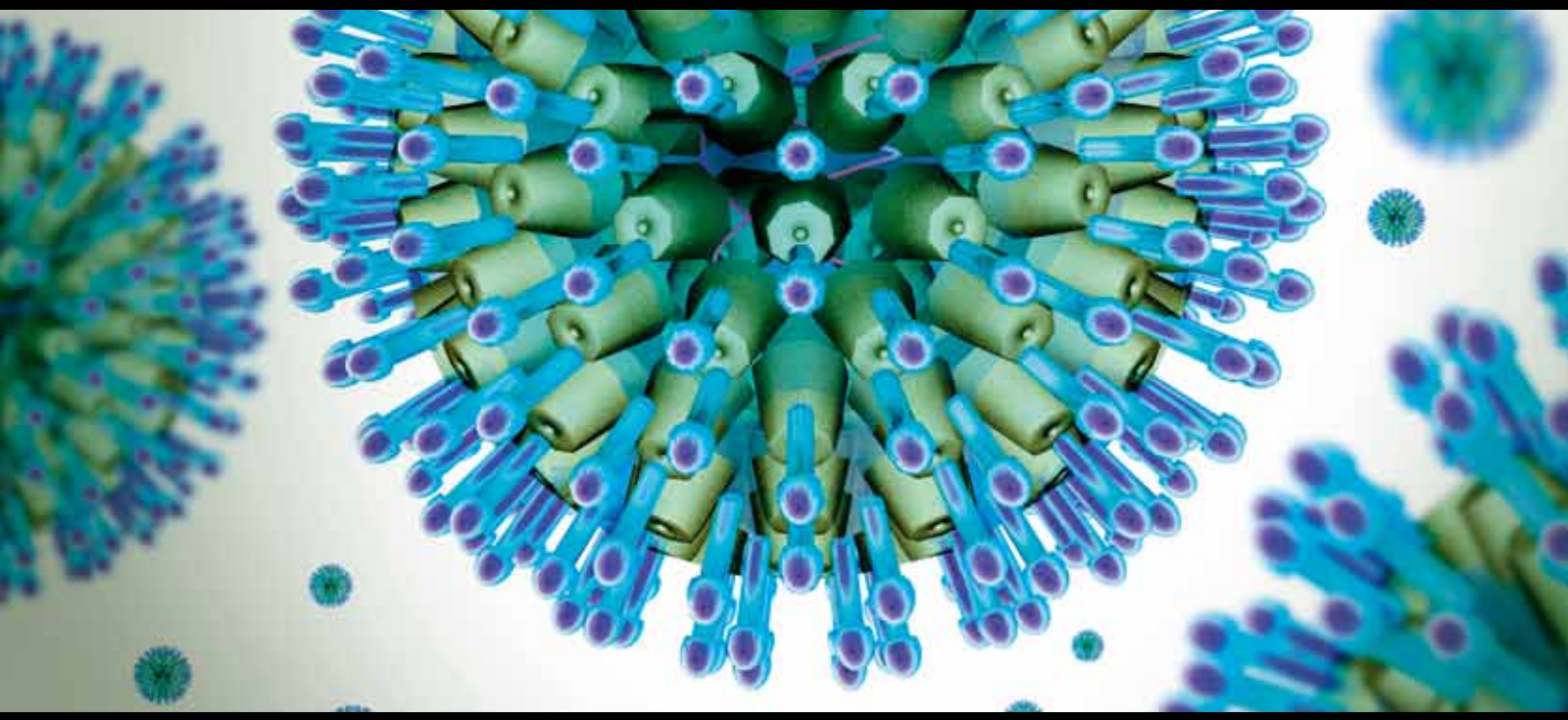
Infectious Diseases in Obstetrics and Gynecology

Pelvic Inflammatory Disease

Guest Editors: Thomas L. Cherpes, Peter A. Rice, and Richard L. Sweet



Pelvic Inflammatory Disease

Pelvic Inflammatory Disease

Guest Editors: Thomas L. Cherpes, Peter A. Rice, and Richard L. Sweet

Editorial Board

Contents

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 714289, 1 page
doi:10.1155/2011/714289

Editorial

Pelvic Inflammatory Disease

Thomas L. Cherpes,[1] Peter A. Rice,[2] and Richard L. Sweet[3]

[1] *Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15224, USA*
[2] *Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA*
[3] *Department of Obstetrics and Gynecology, University of California Davis School of Medicine, Sacramento, CA 95817, USA*

Correspondence should be addressed to Thomas L. Cherpes, cherpestl@upmc.edu

Received 15 December 2011; Accepted 15 December 2011

Pelvic inflammatory disease (PID) is an inflammatory process elicited by the migration of pathogenic microorganisms from the lower to upper genital tract. Although PID is known to increase the risk of tubal factor infertility, many other aspects of this disease remain less well defined. For example, while PID is often caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection, frequently, neither of these bacterial pathogens is isolated from the upper genital tract of women with PID. This etiologic ambiguity also creates uncertainty regarding the decision to include antibiotics effective against genital mycoplasmas and anaerobic vaginal flora in PID treatment. Moreover, since clinical signs and laboratory measurements do not precisely identify all PID cases, and as the accuracy with which imaging modalities identify upper genital tract inflammation is not firmly established, PID remains a diagnostic challenge. It is clear, however, that better understanding of disease pathogenesis, diagnosis, and treatment is needed to improve the care provided women with PID, and this issue of *Infectious Diseases in Obstetrics and Gynecology* was constructed to deliver specific focus on these topics.

Three papers in this issue examine the role of *C. trachomatis* in PID pathogenesis. The first examines findings from *C. trachomatis* infection control programs that have altered our understanding of the host immune response to chlamydial infection, and considers implications of these findings for prophylactic vaccine development. The second paper concisely reviews how non-human primate models of chlamydia infection have improved our understanding of PID pathogenesis, while the third explores a possible association between *C. trachomatis*-specific humoral immunity and genital tract inflammation. The fourth PID pathogenesis-focused paper is a case report that reminds readers of the link between *Actinomyces israelii* and this disease among women using intrauterine devices, while the fifth reviews evidence supporting *Mycoplasma genitalium* as a cause of PID.

The five remaining papers in this issue address PID diagnosis and treatment. The first diagnosis-related paper defines a practical approach for the identification of women with PID, while the second tests an algorithm for PID case identification in epidemiological research using administrative diagnostic codes rather than the more unwieldy medical record review. The third describes an investigation among a cohort of women with high prevalence of HIV-1 that found that the conventional markers of histologic endometritis (i.e., neutrophils and plasma cells) are, at least in this population, unreliable surrogate markers for laparoscopically confirmed salpingitis. The fourth paper reviewed existing literature regarding serologic diagnosis of *C. trachomatis* infection in order to construct an algorithm for chlamydial serologic antibody testing in clinical work-up of the infertile couple. We close this issue with a comprehensive review of the antimicrobial therapies currently available for PID treatment.

Thomas L. Cherpes
Peter A. Rice
Richard L. Sweet

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 959816, 9 pages
doi:10.1155/2011/959816

Review Article

Mycoplasma genitalium: An Emerging Cause of Pelvic Inflammatory Disease

Catherine L. Haggerty[1] and Brandie D. Taylor[2]

[1] *Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA 15261, USA*
[2] *Department of Epidemiology, Michigan State University, East Lansing, MI 48824, USA*

Correspondence should be addressed to Catherine L. Haggerty, haggerty@pitt.edu

Received 14 June 2011; Revised 10 September 2011; Accepted 13 September 2011

Academic Editor: Thomas Cherpes

Mycoplasma genitalium is a sexually transmitted pathogen that is increasingly identified among women with pelvic inflammatory disease (PID). Although *Chlamydia trachomatis* and *Neisseria gonorrhoeae* frequently cause PID, up to 70% of cases have an unidentified etiology. This paper summarizes evidence linking *M. genitalium* to PID and its long-term reproductive sequelae. Several PCR studies have demonstrated that *M. genitalium* is associated with PID, independent of gonococcal and chlamydial infection. Most have been cross-sectional, although one prospective investigation suggested that *M. genitalium* was associated with over a thirteenfold risk of endometritis. Further, a nested case-control posttermination study demonstrated a sixfold increased risk of PID among *M. genitalium* positive patients. Whether or not *M. genitalium* upper genital tract infection results in long-term reproductive morbidity is unclear, although tubal factor infertility patients have been found to have elevated *M. genitalium* antibodies. Several lines of evidence suggest that *M. genitalium* is likely resistant to many frequently used PID treatment regimens. Correspondingly, *M. genitalium* has been associated with treatment failure following cefoxitin and doxycycline treatment for clinically suspected PID. Collectively, strong evidence suggests that *M. genitalium* is associated with PID. Further study of *M. genitalium* upper genital tract infection diagnosis, treatment and long-term sequelae is warranted.

1. Introduction

Mycoplasma genitalium is a genital tract microorganism [1, 2] identified in approximately 15 to 20% of young women seen in some adolescent health centers, sexually transmitted infection clinics, and emergency departments in the United States [3–6]. Concordance of *M. genitalium* infection [1, 2, 7, 8] as well as *M. genitalium* sequence type [9] among sexual partners suggests that this bacteria is sexually transmitted. In some populations studied, infection with *M. genitalium* is as common as *Chlamydia trachomatis* among high risk sexually active women [3, 10] and women with clinically suspected pelvic inflammatory disease (PID) [4]. As *C. trachomatis* is the most common reportable bacterial infection in the United States [11], *M. genitalium* is thus a relatively common infection. *M. genitalium* has been associated with cervicitis [2, 12–15] and may play a role in PID, the infection and inflammation of a woman's upper genital tract [16].

PID is frequent among women of childbearing age, diagnosed in approximately 8% of US women and 15% of Swedish women in their lifetime, with over one million U.S. women treated annually [17–22]. Major reproductive and gynecologic morbidities result from PID, including infertility, ectopic pregnancy, chronic pelvic pain, and recurrent PID [23]. Although PID has a polymicrobial etiology, with *C. trachomatis* and/or *N. gonorrhoeae* isolated from approximately one-third to one half of cases [5, 24–27], many PID cases have an unidentified etiology. Although bacterial vaginosis-associated and mycoplasmal organisms have been associated with PID [4–6, 13, 25, 27–32], independent of gonococcal and chlamydial infection [4, 28], less is known about the etiology, treatment, and sequelae of nongonococcal, nonchlamydial PID. This paper reviews recent evidence for the role of *M. genitalium* in PID and subsequent reproductive and gynecologic outcomes.

2. *Mycoplasma genitalium* Lower Genital Tract Infection

M. genitalium was first identified in the early 1980s among men with nongonococcal urethritis [33]. Because the microbe is extremely difficult to culture, only with polymerase chain reaction (PCR) technology has research into the pathogenicity of *M. genitalium* progressed. Numerous studies have confirmed the role of *M. genitalium* in acute and chronic drug-resistant nongonococcal urethritis [34–36]. In women, *M. genitalium* has been positively associated with cervical inflammation and clinically diagnosed cervicitis, although variable case definitions of cervicitis are responsible for some discrepancies in this literature [12]. As *C. trachomatis* is a common cause of cervicitis and thus may confound this series of studies, some have excluded patients testing positive for *C. trachomatis* or have adjusted for it in multivariate analyses. The vast majority of these have demonstrated an independent, significant association between *M. genitalium* and cervicitis [12].

3. *Mycoplasma genitalium* and PID

PID typically occurs as microorganisms ascend from the lower genital tract and through the cervical os, infecting the uterus, fallopian tubes, and ovaries. Thus, cervicitis is a common antecedent of PID. Because *M. genitalium* is associated with cervicitis [2, 13–15], it is reasonable that it also causes nongonococcal, nonchlamydial PID. Indeed, this organism induces salpingitis in monkeys [37, 38], has been found to ascend from the lower to the upper genital tract in a mouse model [39], causes morphologic changes in ciliated fallopian tube cells in vitro [40], and has been detected in fallopian tube tissue in a woman with salpingitis [41]. Further, *M. genitalium* has been shown to adhere to human spermatozoa, and therefore may potentially be carried by motile sperm to the female upper genital tract [42].

M. genitalium is detected by PCR frequently among women with PID, with rates ranging from 13% to 16% [4, 6, 43]. Several epidemiologic studies have associated *M. genitalium* with clinically suspected PID, endometritis, and adnexitis (see Table 1) [4, 6, 13, 32, 41, 43, 45]. In particular, a handful of studies have examined the relationship between *M. genitalium* identified by PCR and either histologically confirmed endometritis or salpingitis among a population of women with clinically suspected PID [4, 6]. In a study of 115 women presenting to a sexually transmitted disease clinic in Nairobi, Kenya, women with histologically confirmed endometritis were significantly more likely to have *M. genitalium* identified by PCR from the cervix and/or endometrium (16% versus 2%, $P = 0.02$) [6]. After excluding women with gonococcal or chlamydial infection, this study demonstrated an independent association between *M. genitalium* and PID [6]. Similarly, in the PEACH study, Haggerty et al. reported that 15% (88) of 586 women with clinically suspected PID tested positive for *M. genitalium* in the cervix and/or endometrium by PCR. These women were more than twice as likely to have histologically confirmed endometritis at baseline (OR 2.6, 95% CI 1.5–4.6) as compared to women without *M. genitalium* identified at either site, and this relationship remained significant after adjustment for age, race, and gonococcal and chlamydial infection (adjusted OR 2.0, 95% CI 1.0–4.2) [4].

A weakness of the above investigations and a problem which challenges many PID studies are the lack of a true comparison group without signs and symptoms of PID. That is, the control groups were comprised of women with clinically suspected PID who did not have histologically confirmed endometritis. In an attempt to overcome this limitation, a few studies have been conducted with control groups comprised of women without clinically suspected PID. In a study of 53 patients with PID and 80 asymptomatic pregnant women recruited from an obstetrics and gynecology clinic, Uno et al. demonstrated a higher prevalence of *M. genitalium* detected by PCR among the women with PID as compared to controls (6% versus 0%) [13]. In another study of 45 patients with clinically suspected PID and 37 control women undergoing tubal ligation, *M. genitalium* was detected by PCR in 13% of cases versus 0% of controls [43]. These studies collectively demonstrate a higher prevalence of *M. genitalium* among PID patients as compared to external controls, but are limited by the lack of upper genital tract sampling. One study of 194 patients with clinically suspected PID and 246 asymptomatic pregnant women being screened for rubella compared the seroprevalence of *M. genitalium* using a lipid-associated membrane protein-enzyme immunoassay (LAMP-EIA) [44]. Before and after adjustment for chlamydial antibodies, *M. genitalium* was not associated with PID (OR 1.0, 95% CI 0.6–1.7). The null association may be explained by the use of a serologic marker of *M. genitalium*, which measures both acute and past exposure. Thus, it may be that only current or recent *M. genitalium* infection is associated with current PID.

The cross-sectional nature of most *M. genitalium* and PID studies has made it difficult to determine whether or not the relationship is causal. However, there are a handful of prospective studies which allow for temporal assessment. Within the PEACH cohort, Haggerty et al. demonstrated that the relationship between *M. genitalium* and endometritis was independent and causal, since among women without concurrent *N. gonorrhoeae* and/or *C. trachomatis*, a positive endometrial PCR test for *M. genitalium* was associated with over a thirteenfold risk of incident endometritis, assessed histologically 30 days following a baseline evaluation of *M. genitalium* (adjusted RR 13.4, 2.4–75.2) [4]. Similarly, in a nested case-control study of 2079 women presenting for pregnancy termination at Malmo University Hospital, *M. genitalium* was significantly associated with postabortal PID (OR 6.3, 95% CI 1.6–25.2) [45]. Lastly, a study of 2378 sexually active female students participating in a chlamydia screening trial in London reported a positive, nonsignificant association between *M. genitalium* and subsequent PID (RR 2.4, 95% CI 0.7–7.5) [46]. There are several reasons why this study's findings are different from those by Haggerty and Bjartling. First, despite the large sample size, the study was underpowered to detect a prospective association between *M. genitalium* and PID. Second, PID was assessed largely by

Table 1: Studies evaluating the relationship between *M. genitalium* and pelvic inflammatory disease.

Citation	Sample size, population, setting	Study design	Methods: *M. genitalium* test PID diagnosis	Findings	Validity
Uno et al. [13]	200 patients aged 19 to 49 years visiting the OB Gyn department of Kizawa Memorial Hospital and Hayasaki Ladies Clinic.	Cross-sectional	*M. genitalium*: PCR of endocervical specimens. PID: clinical criteria.	5.7% of PID patients versus 0% of pregnant controls tested positive for *M. genitalium* (*P*-value or OR not reported, one patient co-infected with *C. trachomatis*).	Strengths: Control group of patients without signs and symptoms of PID. Limitations: No laparoscopic or histologic confirmation of PID. Although *C. trachomatis* was assessed, sample size too small to determine independent effect of *M. genitalium*
Cohen et al. [6]	115 patients presenting with pelvic pain ≤ 14 days presenting to a sexually transmitted diseases clinic, Nairobi, Kenya between 2000–2003.	Cross-sectional	*M. genitalium:* PCR of cervical and endometrial samples. PID**:** histologically confirmed endometritis.	*M. genitalium* detected in 16% of patients with endometritis versus 2% of patients without endometritis ($P = 0.03$). *M. genitalium* identified in the endometrium was associated with endometritis after excluding women with gonococcal or chlamydial infection ($P = 0.03$, percentages not presented in the paper).	Strengths: PID defined histologically. Adjustment for *C. trachomatis* and *N. gonorrhoeae* allows for independent association between *M. genitalium* and PID to be examined. Limitations: No control group of women without clinically suspected PID. Cross-sectional design does not allow for a temporal association to be proven
Simms et al. [43]	45 patients with a clinical diagnosis of PID (ages 16–43) and 37 patients undergoing tubal ligation (ages 21–45).	Case-control study	*M. genitalium:* PCR of endocervical swabs. PID: clinical criteria.	*M. genitalium* detected in 13% of patients versus 0% of controls.	Strengths: Control group of patients without signs and symptoms of PID (although not confirmed histologically or laparoscopically. Limitations: No upper genital tract specimens collected. PID was not confirmed laparoscopically or histologically. No adjustment for confounders. Cross-sectional design does not allow for a temporal association to be proven.
Cohen et al. [41]	123 women aged 18–40 with laparoscopically confirmed PID treated at Kenyatta National Hospital, 2000–2003.	Cross-sectional study	*M. genitalium*: PCR of cervical, endometrial, and fallopian tube samples. PID: laparoscopically diagnosed salpingitis, graded as mild, moderate, or severe.	*M. genitalium* detected in the fallopian tube of one patient. 6% of women with mild, 11% of women with moderate, and 6% of women with severe salpingitis tested positive for *M. genitalium* in one or more site.	Strengths: PID verified by laparoscopy. Limitations: No control group of women without PID. Cross-sectional design does not allow for a temporal association to be proven.

Table 1: Continued.

Citation	Sample size, population, setting	Study design	Methods: *M. genitalium* test PID diagnosis	Findings	Validity
Jurstrand et al. [44]	194 inpatients with PID aged 15–50 and 83 inpatients with ectopic pregnancy (EP) aged 18–42 treated in the OBGyn department of Örebo University Hospital, Örebo, Sweden, 1984–1986. 246 healthy pregnant women being screened for rubella were matched to ectopic pregnancy cases by age.	Case control study	*M. genitalium*: antibodies assessed using a lipid-associated membrane protein-enzyme immunoassay (LAMP-EIA). PID: clinical criteria.	*M. genitalium* and PID: Crude OR 1.3 (0.7–2.2). Adjusted OR 1.0 (0.6–1.7) *M. genitalium* & EP: Crude OR 1.3 (0.7–2.5). Adjusted OR 1.0 (0.5–2.0). (Adjusted for age and *C. trachomatis* antibodies.)	Strengths: LAMP-EIA covers antigenic variation of different genotypes of *M. genitalium* with no cross-reactivity with other Mycoplasma species. Limitations: PID not laparoscopically or histologically confirmed. Limited adjustment for confounders. Unable to determine timing of *M. genitalium* infection in relation to the acute PID episode.
Haggerty et al. [4]	682 women with clinically suspected PID aged 14–37 years recruited from ER, OB/Gyn, STD clinics, and private practice from 13 U.S. urban clinical sites, 1996–1999.	Prospective	*M. genitalium:* PCR of cervical and endometrial samples. PID: histologically confirmed endometritis assessed at baseline and at a 30-day follow-up clinic visit.	Baseline comparison of *M. genitalium* (endometrium) and endometritis: Adjusted OR 3.0 (1.5–6.1). Prospective evaluation of baseline *M. genitalium* and incident endometritis (30-days follow-up visit): Adjusted RR 13.4 (2.4–75.2). (Adjusted for age, race, *C. trachomatis*, and *N. gonorrhoeae.*)	Strengths: Large sample size. Histologic confirmation of PID. Prospective analysis of baseline *M. genitalium* infection and incident endometritis at the 30 day follow-up visit supports a temporal association. Adjustment for *C. trachomatis* and *N. gonorrhoeae* allows for independent association between *M. genitalium* and PID to be examined. Limitations: No control group of women without clinically suspected PID
Bjartling et al. [45]	2079 women aged 15–40 presenting for termination of pregnancy at Malmö University Hospital, Sweden, 2003–2007.	Prospective	*M. genitalium:* PCR of urine, vaginal, and cervical samples. PID: clinical criteria.	*M. genitalium* & posttermination PID: Adjusted OR 6.3 (1.6–25.2). (Adjusted for age and *C. trachomatis.*)	Strengths: Prospective design allows for temporal inference. Adjustment for *C. trachomatis* allows for independent association between *M. genitalium* and PID to be examined. Limitations: Generalizability limited to post-abortal PID. No upper genital tract specimens tested. PID not confirmed laparoscopically or histologically.

Table 1: Continued.

Citation	Sample size, population, setting	Study design	Methods: *M. genitalium* test PID diagnosis	Findings	Validity
Oakeshott et al. [46]	2378 sexually active female students (mean age 21 years) participating in a chlamydia screening trial, London, 2004–2006.	Prospective	*M. genitalium*: PCR of self-collected vaginal swabs. PID: Self-reported PID and PID symptoms, and medical records/clinical diagnosis for a subset of women not completing follow-up questionnaire, assessed over 12 months.	*M. genitalium* & PID: RR 2.4 (0.7–7.5).	Strengths. Large sample size. Prospective design allows a temporal relationship to be explored. Limitations: PID diagnosis based on self-report and limited medical record/clinical diagnosis; no laparoscopic or histologic confirmation. Asymptomatic PID not captured. Despite large sample, the study was underpowered to detect a prospective association between *M. genitalium* and PID. Selection bias may have caused an underestimate of *M. genitalium.*

self-report and thus may suffer from misclassification bias. Further, asymptomatic PID could not be captured in this study. Additional prospective studies with active surveillance of PID using biologic markers are needed to fully understand the relationship between *M. genitalium* and PID.

4. Does *M. genitalium* Infection Result in Long-Term Reproductive Morbidity?

PID may result in long-term reproductive sequelae, including infertility, ectopic pregnancy, and chronic pelvic pain. Evidence for this comes from the Lund, Sweden cohort study (1960–1984) in which among 2,501 women with clinically suspected PID, salpingitis verified by laparoscopy was associated with infertility, ectopic pregnancy, recurrent PID, and chronic pelvic pain [23, 47]. Additionally, a number of retrospective case-control studies have shown that women with tubal occlusion are more likely to bear chlamydial or gonococcal antibodies, providing human evidence for causal links between chlamydial PID, gonococcal PID, and infertility [48–52].

Whether or not *M. genitalium* upper genital tract infection can result in reproductive or gynecologic sequelae is unclear. Like *C. trachomatis*, *M. genitalium* is often asymptomatic [1], increasing the likelihood for "silent" PID and its sequelae. Also parallel to studies of *C. trachomatis*, *M. genitalium* antibodies have been identified more frequently (22% versus 6%) among 132 women with tubal factor infertility compared to 176 women nontubal factor infertility [53]. In a subsequent serologic investigation of *M. genitalium* and tubal factor infertility by the same investigator, 212 couples attending fertility clinics were examined and a strong antibody response against *M. genitalium* or *C. trachomatis*, but no sign of current or chronic infection, was found in women with TFI, indicating that previous infections caused by these microorganisms may have resulted in permanent damage and occlusion of the fallopian tubes [54]. In another study of 51 infertility patients and 23 healthy, fertile women, *M. genitalium* was identified in the cervical canal by PCR among 20% of cases versus 4% of controls ($P = 0.16$) [55]. In subgroup analyses, *M. genitalium* was found in 29% (7 of 24) women with idiopathic infertility, and the comparison to controls was of borderline statistical significance ($P = 0.05$). Although these relationships were not statistically significant, they suggest that current infection with *M. genitalium* and/or permanent damage to the reproductive tract caused by chronic infection with *M. genitalium* may impair fertility. One study has examined the relationship between *M. genitalium* and reproductive morbidity among a population of women with PID. In an analysis of 586 women from the PEACH study presenting with signs and symptoms of PID, Haggerty et al. reported that rates of sequelae, including chronic pelvic pain (42%), infertility (22%), and recurrent PID (31%), were high among women testing positive for active endometrial *M. genitalium* by PCR at baseline [4]. Although differences in rates of sequelae were not significantly different between women testing positive or negative for *M. genitalium*, there was a trend toward increased chronic pelvic pain, infertility, and recurrent PID and decreased pregnancy and live birth following *M. genitalium* infection. The rate of subsequent infertility among women with active endometrial *M. genitalium* was approximately twice as high as the rate reported from a study utilizing the

2002 National Survey of Family Growth data [17], suggesting that preservation of fertility may be suboptimal for women with *M. genitalium* upper genital tract infection.

Data examining *M. genitalium* and other reproductive consequences are sparse. One serologic case-control study of 82 ectopic pregnancy cases and 246 healthy pregnancy control women found no statistically significant association between ectopic pregnancy and *M. genitalium* antibodies [44]. Nonsignificant trends suggesting an association between *M. genitalium* and ectopic pregnancy were found among a subgroup of women aged 18–30 (OR 2.0, $P = 0.133$) and among women testing negative for *C. trachomatis* antibodies (OR 2.3, $P = 0.161$) [44]. It may be possible that reduced power in these subset analyses limited the ability to detect statistically significant associations. Further large prospective studies utilizing both serology and PCR are needed to better understand the potential reproductive sequelae of *M. genitalium* infection.

5. Symptoms of *M. genitalium* and Implications for Delayed Treatment

Although some studies have linked *M. genitalium* to pathologic vaginal discharge [56] and urethritis [57], several have reported that both *M. genitalium* [7, 58, 59] and *C. trachomatis* [60] are comparatively less symptomatic than gonococcal infection [60]. Harboring an asymptomatic infection may increase the likelihood for delayed care and development of sequelae. In a study of 516 sexual dyads, although *M. genitalium* was associated with urethral discharge in men, no symptoms were diagnostic of infection in women [8]. In addition, *M. genitalium* was found to be common in asymptomatic patients attending an STD clinic in the United Kingdom [61].

Symptoms of PID vary by microbial pathogen. For example, chlamydial salpingitis tends to exhibit more mild symptoms than gonococcal PID, despite the fact that both pathogens cause tubal damage [60]. Short et al. found that, compared to women with gonococcal PID, those with *M. genitalium*-associated PID were less likely to have elevated markers of inflammation, cervicitis, elevated vaginal pH, and a high pelvic pain score [58]. However, signs and symptoms of PID were similar between women with *C. trachomatis* and *M. genitalium* [58]. This may indicate that, among women with PID, those infected with *N. gonorrhoeae* present with more overt and severe symptoms, leading to earlier treatment than women with *C. trachomatis* or *M. genitalium* [60]. Long time to treatment is a major concern, as a case-control study nested within a landmark Scandinavian study found that delaying care for 3 or more days significantly increased the risk of impaired fertility among 443 women with PID [62]. In a more recent study of 298 women with histologically confirmed endometritis, those with *C. trachomatis* monoinfection and *M. genitalium* monoinfection reported waiting the longest time between onset of symptoms and care seeking (12.3 and 10.9 days), while the shortest times were among women with *N. gonorrhoeae* monoinfection (4.6 days) and coinfection with two or more pathogens (5.6 days) [63]. Delayed treatment of PID for 14 days or more was not significantly associated with reproductive morbidity in this study. However, rates of infertility, recurrent PID, and chronic pelvic pain were high in this cohort (17%, 20%, and 36%). Collectively, these studies may suggest that women with *M. genitalium*-associated PID may have low levels of chronic inflammation that can lead to reproductive damage before treatment.

6. Treatment of Upper Genital Tract *M. genitalium* Infection

If women with *M. genitalium* upper genital tract infection do seek care, they will likely be treated with one of the currently recommended CDC treatment regimens for PID including (1) ofloxacin, (2) levofloxacin, (3) ceftriaxone plus doxycycline, or (4) cefoxitin and probenecid plus doxycycline; all with optional metronidazole for full coverage against anaerobes and BV [64]. However, some of these regimens are ineffective for the treatment of *M. genitalium*. In the PEACH study, Haggerty et al reported that persistence of *M. genitalium* was very high among women treated with cefoxitin and doxycycline for PID, with 44% of women with baseline endometrial PCR-positive specimens testing positive again 30 days following treatment [4]. In contrast, only 2% to 4% of women in the PEACH study had persistent or recurrent gonococcal or chlamydial cervicitis when retested at 30 days [24]. Women with *M. genitalium* identified in the endometrium by PCR at study enrollment were four times as likely to experience persistent endometritis and over four times as likely to experience treatment failure, defined as the presence of both endometritis and pelvic pain 30 days following treatment for PID (adjusted RR 4.6, 95% CI 1.1–20.1) [4]. Further, *M. genitalium* strains resistant to tetracycline have been isolated [65], and *M. genitalium* is associated with persistent nongonococcal urethritis among men treated with tetracyclines [35, 59, 66–68] and levofloxacin [69, 70] for nongonococcal urethritis. Thus, even if women with active *M. genitalium* upper genital tract infection seek treatment, antibiotic resistance among *M. genitalium* strains may lead to persistent or recurrent infection, resulting in chronic inflammation and infection.

7. Conclusion

PID is a common disease among American women that results in frequent, serious reproductive morbidity. Most women with PID are treated with antibiotics directed toward *N. gonorrhoeae* and/or *C. trachomatis*, despite the fact that these bacterial pathogens account for only a third to a half of PID cases. Although *M. genitalium* has recently been recognized as a cause of nongonococcal, nonchlamydial PID, little is known about the long-term prognosis of *M. genitalium* upper genital tract infection.

Given the scarcity of information regarding the long-term prognosis of women infected with *M. genitalium*, the lack of routine testing for *M. genitalium* in clinical practice, and the resistance of *M. genitalium* to a number of PID

treatment regimens, additional research on the relationships between *M. genitalium*, PID, and long-term reproductive sequelae is critically needed in order to shape screening and treatment guidelines. The high rate of treatment failure among women with clinically suspected PID testing positive for *M. genitalium* emphasizes a need for PID antibiotic regimens targeted toward *M. genitalium*, with the ultimate goal to prevent reproductive and gynecologic morbidity. *M. genitalium* has demonstrated susceptibility to macrolides, with azithromycin being the most active, and variable resistance to fluroquinolones, including ciprofloxacin [36, 71]. However, it should be noted that *M. genitalium* azithromycin resistance has recently been reported [72, 73]. A newer quinolone, moxiflocacin, has recently been shown to exhibit a high degree of activity against *M. genitalium* [74], and this antibiotic has also been shown to be effective for the treatment of PID [75]. Although these promising therapies warrant further study for the treatment of PID, no highly sensitive test is widely used to diagnose *M. genitalium* in clinical practice. Nucleic acid amplification tests (NAATs) have been developed and tested [76], and they may be useful for the clinical detection of *M. genitalium* among PID patients. Endocervical swabs collected from patients with clinically suspected PID are already often tested for gonococcal and chlamydial infection, and thus a NAAT for *M. genitalium* could efficiently be added to this diagnostic screening. *M. genitalium* screening among patients with clinically suspected PID would allow clinicians to select treatment regimens specific for mycoplasmal PID. Additionally, commercially available testing is also critical for the identification and treatment of uncomplicated lower genital tract *M. genitalium* infection, in order to prevent subsequent PID and potential sequelae.

References

[1] J. Williams, B. van der Pol, B. E. Batteiger et al., "*Mycoplasma genitalium* symptoms and concordance in sexual dyads," *International Society for STD Research Book of Abstracts*, 2007.

[2] L. Falk, H. Fredlund, and J. S. Jensen, "Signs and symptoms of urethritis and cervicitis among women with or without *Mycoplasma genitalium* or *Chlamydia trachomatis* infection," *Sexually Transmitted Infections*, vol. 81, no. 1, pp. 73–78, 2005.

[3] J. S. Huppert, J. E. Mortensen, J. L. Reed, J. A. Kahn, K. D. Rich, and M. M. Hobbs, "*Mycoplasma genitalium* detected by transcription-mediated amplification is associated with *Chlamydia trachomatis* in adolescent women," *Sexually Transmitted Diseases*, vol. 35, no. 3, pp. 250–254, 2008.

[4] C. L. Haggerty, P. A. Totten, S. G. Astete et al., "Failure of cefoxitin and doxycycline to eradicate endometrial *Mycoplasma genitalium* and the consequence for clinical cure of pelvic inflammatory disease," *Sexually Transmitted Infections*, vol. 84, no. 5, pp. 338–342, 2008.

[5] I. Simms, K. Eastick, H. Mallinson et al., "Associations between *Mycoplasma genitalium*, *Chlamydia trachomatis* and pelvic inflammatory disease," *Journal of Clinical Pathology*, vol. 56, no. 8, pp. 616–618, 2003.

[6] C. R. Cohen, L. E. Manhart, E. A. Bukusi et al., "Association bet- ween *Mycoplasma genitalium* and acute endometritis," *Lancet*, vol. 359, no. 9308, pp. 765–766, 2002.

[7] A. K. Tosh, B. van der Pol, J. D. Fortenberry et al., "*Mycoplasma genitalium* among Adolescent Women and their Partners," *Journal of Adolescent Health*, vol. 40, no. 5, pp. 412–417, 2007.

[8] A. R. Thurman, O. Musatovova, S. Perdue, R. N. Shain, J. G. Baseman, and J. B. Baseman, "*Mycoplasma genitalium* symptoms, concordance and treatment in high-risk sexual dyads," *International Journal of STD and AIDS*, vol. 21, no. 3, pp. 177–183, 2010.

[9] S. V. Hjorth, E. Björnelius, P. Lidbrink et al., "Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission," *Journal of Clinical Microbiology*, vol. 44, no. 6, pp. 2078–2083, 2006.

[10] C. Gaydos, N. E. Maldeis, A. Hardick, J. Hardick, and T. C. Quinn, "*Mycoplasma genitalium* as a contributor to the multiple etiologies of cervicitis in women attending sexually transmitted disease clinics," *Sexually Transmitted Diseases*, vol. 36, no. 10, pp. 598–606, 2009.

[11] Centers for Disease Control and Prevention, *Sexually Transmitted Disease Surveillance, 2004*, U.S. Department of Health and Human Services, Atlanta, Ga, USA, 2005.

[12] C. L. McGowin and C. Anderson-Smits, "*Mycoplasma genitalium*: an emerging cause of sexually transmitted disease in women," *PLoS Pathogens*, vol. 7, no. 5, pp. 1–10, 2011.

[13] M. Uno, T. Deguchi, H. Komeda et al., "*Mycoplasma genitalium* in the cervices of Japanese women," *Sexually Transmitted Diseases*, vol. 24, no. 5, pp. 284–286, 1997.

[14] L. E. Manhart, C. W. Critchlow, K. K. Holmes et al., "Mucopurulent cervicitis and *Mycoplasma genitalium*," *Journal of Infectious Diseases*, vol. 187, no. 4, pp. 650–657, 2003.

[15] J. Pépin, A. C. Labbé, N. Khonde et al., "*Mycoplasma genitalium*: an organism commonly associated with cervicitis among west African sex workers," *Sexually Transmitted Infections*, vol. 81, no. 1, pp. 67–72, 2005.

[16] C. L. Haggerty and R. B. Ness, "Epidemiology, pathogenesis and treatment of pelvic inflammatory disease," *Expert Review of Anti-Infective Therapy*, vol. 4, no. 2, pp. 235–247, 2006.

[17] E. H. Stephen and A. Chandra, "Declining estimates of infertility in the United States: 1982–2002," *Fertility and Sterility*, vol. 86, no. 3, pp. 516–523, 2006.

[18] Centers for Disease Control and Prevention, *Sexually Transmitted Disease Surveillance, 1997*, Division of STD Prevention, U.S. Department of Health and Human Services, Atlanta, Ga, USA, 1998.

[19] A. E. Washington and P. Katz, "Cost of and payment source for pelvic inflammatory disease: trends and projections, 1983 through 2000," *Journal of the American Medical Association*, vol. 266, no. 18, pp. 2565–2569, 1991.

[20] D. B. Rein, W. J. Kassler, K. L. Irwin, and L. Rabiee, "Direct medical cost of pelvic inflammatory disease and its sequelae: decreasing, but still substantial," *Obstetrics and Gynecology*, vol. 95, no. 3, pp. 397–402, 2000.

[21] L. Westrom, "Decrease in incidence of women treated in hospital for acute salpingitis in Sweden," *Genitourinary Medicine*, vol. 64, no. 1, pp. 59–63, 1988.

[22] I. Simms, P. Rogers, and A. Charlett, "The rate of diagnosis and demography of pelvic inflammatory disease in general practice: England and Wales," *International Journal of STD and AIDS*, vol. 10, no. 7, pp. 448–451, 1999.

[23] L. Westrom, R. Joesoef, G. Reynolds, A. Hagdu, and S. E. Thompson, "Pelvic inflammatory disease and fertility: a cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results," *Sexually Transmitted Diseases*, vol. 19, no. 4, pp. 185–192, 1992.

[24] R. B. Ness, D. E. Soper, R. L. Holley et al., "Effectiveness of inpatient and outpatient treatment strategies for women with pelvic inflammatory disease: results from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) randomized trial," *American Journal of Obstetrics and Gynecology*, vol. 186, no. 5, pp. 929–937, 2002.

[25] S. L. Hillier, N. B. Kiviat, S. E. Hawes et al., "Role of bacterial vaginosis-associated microorganisms in endometritis," *American Journal of Obstetrics and Gynecology*, vol. 175, no. 2, pp. 435–441, 1996.

[26] N. B. Kiviat, P. Wolner-Hanssen, D. A. Eschenbach et al., "Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis," *American Journal of Surgical Pathology*, vol. 14, no. 2, pp. 167–175, 1990.

[27] D. E. Soper, N. J. Brockwell, H. P. Dalton, and D. Johnson, "Observations concerning the microbial etiology of acute salpingitis," *American Journal of Obstetrics and Gynecology*, vol. 170, no. 4, pp. 1008–1017, 1994.

[28] C. L. Haggerty, S. L. Hillier, D. C. Bass, and R. B. Ness, "Bacterial vaginosis and anaerobic bacteria are associated with endometritis," *Clinical Infectious Diseases*, vol. 39, no. 7, pp. 990–995, 2004.

[29] J. F. Peipert, A. B. Montagno, A. S. Cooper, and C. J. Sung, "Bacterial vaginosis as a risk factor for upper genital tract infection," *American Journal of Obstetrics and Gynecology*, vol. 177, no. 5, pp. 1184–1187, 1997.

[30] R. P. Nugent, M. A. Krohn, and S. L. Hillier, "Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation," *Journal of Clinical Microbiology*, vol. 29, no. 2, pp. 297–301, 1991.

[31] H. C. Wiesenfeld, S. L. Hillier, M. A. Krohn et al., "Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 100, no. 3, pp. 456–463, 2002.

[32] C. L. Haggerty, P. A. Totten, S. G. Astete, and R. B. Ness, "Mycoplasma genitalium among women with nongonococcal, nonchlamydial pelvic inflammatory disease," *Infectious Diseases in Obstetrics and Gynecology*, vol. 2006, Article ID 30184, 5 pages, 2006.

[33] J. G. Tully, D. Taylor-Robinson, R. M. Cole, and D. L. Rose, "A newly discovered mycoplasma in the human urogenital tract," *Lancet*, vol. 1, no. 8233, pp. 1288–1291, 1981.

[34] P. A. Totten, M. A. Schwartz, K. E. Sjörström et al., "Association of *Mycoplasma genitalium* with nongonococcal urethritis in heterosexual men," *Journal of Infectious Diseases*, vol. 183, no. 2, pp. 269–276, 2001.

[35] J. S. Jensen, "*Mycoplasma genitalium*: a cause of nongonococcal urethritis?" *Genitourinary Medicine*, vol. 70, no. 5, p. 363, 1994.

[36] D. Taylor-Robinson, "*Mycoplasma genitalium*—an up-date," *International Journal of STD and AIDS*, vol. 13, no. 3, pp. 145–151, 2002.

[37] B. R. Moller, D. Taylor-Robinson, P. M. Furr, and E. A. Freundt, "Acute upper genital-tract disease in female monkeys provoked experimentally by *Mycoplasma genitalium*," *British Journal of Experimental Pathology*, vol. 66, no. 4, pp. 417–426, 1985.

[38] D. Taylor-Robinson, P. M. Furr, J. G. Tully, M. F. Barile, and B. R. Møller, "Animal models of *Mycoplasma genitalium* urogenital infection," *Israel Journal of Medical Sciences*, vol. 23, no. 6, pp. 561–564, 1987.

[39] C. L. McGowin, R. A. Spagnuolo, and R. B. Pyles, "*Mycoplasma genitalium* rapidly disseminates to the upper reproductive tracts and knees of female mice following vaginal inoculation," *Infection and Immunity*, vol. 78, no. 2, pp. 726–736, 2010.

[40] A. Baczynska, P. Funch, J. Fedder, H. J. Knudsen, S. Birkelund, and G. Christiansen, "Morphology of human Fallopian tubes after infection with *Mycoplasma genitalium* and Mycoplasma hominis—n vitro organ culture study," *Human Reproduction*, vol. 22, no. 4, pp. 968–979, 2007.

[41] C. R. Cohen, N. R. Mugo, S. G. Astete et al., "Detection of *Mycoplasma genitalium* in women with laparoscopically diagnosed acute salpingitis," *Sexually Transmitted Infections*, vol. 81, no. 6, pp. 463–466, 2005.

[42] H. F. Svenstrup, J. Fedder, J. Abraham-Peskir, S. Birkelund, and G. Christiansen, "*Mycoplasma genitalium* attaches to human spermatozoa," *Human Reproduction*, vol. 18, no. 10, pp. 2103–2109, 2003.

[43] I. Simms, J. M. Stephenson, H. Mallinson et al., "Risk factors associated with pelvic inflammatory disease," *Sexually Transmitted Infections*, vol. 13, no. 18, 2002.

[44] M. Jurstrand, J. S. Jensen, A. Magnuson, F. Kamwendo, and H. Fredlund, "A serological study of the role of *Mycoplasma genitalium* in pelvic inflammatory disease and ectopic pregnancy," *Sexually Transmitted Infections*, vol. 83, no. 4, pp. 319–323, 2007.

[45] C. Bjartling, S. Osser, and K. Persson, "The association between *Mycoplasma genitalium* and pelvic inflammatory disease after termination of pregnancy," *An International Journal of Obstetrics and Gynaecology*, vol. 117, no. 3, pp. 361–364, 2010.

[46] P. Oakeshott, A. Aghaizu, P. Hay et al., "Is *Mycoplasma genitalium* in women the "new chlamydia?" A community-based prospective cohort study," *Clinical Infectious Diseases*, vol. 51, no. 10, pp. 1160–1166, 2010.

[47] L. Westrom, "Effect of acute pelvic inflammatory disease on fertility," *American Journal of Obstetrics and Gynecology*, vol. 121, no. 5, pp. 707–713, 1975.

[48] R. Punnonen, P. Terho, V. Nikkanen, and O. Meurman, "Chlamydial serology in infertile women by immunofluorescence," *Fertility and Sterility*, vol. 31, no. 6, pp. 656–659, 1979.

[49] J. L. Kane, R. M. Woodland, and T. Forsey, "Evidence of chlamydial infection in infertile women with and without fallopian tube obstruction," *Fertility and Sterility*, vol. 42, no. 6, pp. 843–848, 1984.

[50] A. Miettinen, P. K. Heinonen, K. Teisala, K. Hakkarainen, and R. Punnonen, "Serologic evidence for the role of *Chlamydia trachomatis*, *Neisseria Gonorrhoeae*, and *Mycoplasma hominis* in the etiology of tubal factor infertility and ectopic pregnancy," *Sexually Transmitted Diseases*, vol. 17, no. 1, pp. 10–14, 1990.

[51] S. Chutivongse, M. Kozuh-Novak, J. Annus et al., "Tubal infertility: serologic relationship to past chlamydial and gonococcal infection," *Sexually Transmitted Diseases*, vol. 22, no. 2, pp. 71–77, 1995.

[52] G. Anestad, O. Lunde, M. Moen, and K. Dalaker, "Infertility and chlamydial infection," *Fertility and Sterility*, vol. 48, no. 5, pp. 787–790, 1987.

[53] H. F. Clausen, J. Fedder, M. Drasbek et al., "Serological investigation of *Mycoplasma genitalium* in infertile women," *Human Reproduction*, vol. 16, no. 9, pp. 1866–1874, 2001.

[54] H. F. Svenstrup, J. Fedder, S. E. Kristoffersen, B. Trolle, S. Birkelund, and G. Christiansen, "*Mycoplasma genitalium*,

Chlamydia trachomatis, and tubal factor infertility—a prospective study," *Fertility and Sterility*, vol. 90, no. 3, pp. 513–520, 2008.

[55] J. Grześko, M. Elias, B. Maczyńska, U. Kasprzykowska, M. Tłaczała, and M. Goluda, "Occurrence of *Mycoplasma genitalium* in fertile and infertile women," *Fertility and Sterility*, vol. 91, no. 6, pp. 2376–2380, 2009.

[56] J. E. Korte, J. B. Baseman, M. P. Cagle et al., "Cervicitis and genitourinary symptoms in women culture positive for *Mycoplasma genitalium*," *American Journal of Reproductive Immunology*, vol. 55, no. 4, pp. 265–275, 2006.

[57] H. Moi, N. Reinton, and A. Moghaddam, "*Mycoplasma genitalium* in women with lower genital tract inflammation," *Sexually Transmitted Infections*, vol. 85, no. 1, pp. 10–14, 2009.

[58] V. L. Short, P. A. Totten, R. B. Ness, S. G. Astete, S. F. Kelsey, and C. L. Haggerty, "Clinical presentation of *Mycoplasma genitalium* infection versus *Neisseria Gonorrhoeae* infection among women with pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 48, no. 1, pp. 41–47, 2009.

[59] L. Falk, H. Fredlund, and J. S. Jensen, "Tetracycline treatment does not eradicate *Mycoplasma genitalium*," *Sexually Transmitted Infections*, vol. 79, no. 4, pp. 318–319, 2003.

[60] J. Paavonen, L. Westrom, and D. Escenbah, "Pelvic inflammatory disease," in *Sexually Transmitted Diseases*, K. K. Holmes, P. F. Sparling, W. E. Stamm et al., Eds., pp. 1017–1050, McGraw–Hill, New York, NY, USA, 2008.

[61] J. D. C. Ross, L. Brown, P. Saunders, and S. Alexander, "*Mycoplasma genitalium* in asymptomatic patients: implications for screening," *Sexually Transmitted Infections*, vol. 85, no. 6, pp. 436–437, 2009.

[62] S. D. Hillis, R. Joesoef, P. A. Marchbanks, J. N. Wasserheit, W. Cates, and L. Westrom, "Delayed care of pelvic inflammatory disease as a risk factor for impaired fertility," *American Journal of Obstetrics and Gynecology*, vol. 168, no. 5, pp. 1503–1509, 1993.

[63] B. D. Taylor, R. B. Ness, T. Darville, and C. L. Haggerty, "Microbial correlates of delayed care for pelvic inflammatory disease," *Sexually Transmitted Diseases*, vol. 38, no. 5, pp. 434–438, 2011.

[64] Centers for Disease Control and Prevention, "Sexually transmitted diseases treatment guidelines," *Morbidity and Mortality Weekly Report*, vol. 59, pp. 1–110, 2010.

[65] G. E. Kenny, P. A. Young, M. C. Roberts, L. A. Manhart, and P. A. Totten, "An improved medium for growth of *Mycoplasma genitalium*: isolation of a tetracycline-resistant clinical strain," *ISSTDR Book of Abstracts*, p. 389, 2007.

[66] H. Carlberg, E. Bjornelius, and J. S. Jensen, "*Mycoplasma genitalium*—the search for effective treatment," *International Journal of STD & AIDS*, vol. 13, pp. 30–31, 2002.

[67] G. Johannisson, Y. Enström, G. B. Löwhagen et al., "Occurrence and treatment of *Mycoplasma genitalium* in patients visiting STD clinics in Sweden," *International Journal of STD and AIDS*, vol. 11, no. 5, pp. 324–326, 2000.

[68] P. J. Horner, C. B. Gilroy, B. J. Thomas, R. O. M. Naidoo, and D. Taylor-Robinson, "Association of *Mycoplasma genitalium* with acute non-gonococcal urethritis," *Lancet*, vol. 342, no. 8871, pp. 582–585, 1993.

[69] T. Deguchi, T. Yoshida, S. Yokoi et al., "Longitudinal quantitative detection by real-time PCR of *Mycoplasma genitalium* in first-pass urine of men with recurrent nongonococcal urethritis," *Journal of Clinical Microbiology*, vol. 40, no. 10, pp. 3854–3856, 2002.

[70] S. I. Maeda, M. Tamaki, K. Kojima et al., "Association of *Mycoplasma genitalium* persistence in the urethra with recurrence of nongonococcal urethritis," *Sexually Transmitted Diseases*, vol. 28, no. 8, pp. 472–476, 2001.

[71] P. C. T. Hannan, "Comparative susceptibilities of various AIDS-associated and human urogenital tract mycoplasmas and strains of Mycoplasma pneumoniae to 10 classes of antimicrobial agent in vitro," *Journal of Medical Microbiology*, vol. 47, no. 12, pp. 1115–1122, 1998.

[72] C. S. Bradshaw, M. Y. Chen, and C. K. Fairley, "Persistence of *Mycoplasma genitalium* following azithromycin therapy," *PLoS ONE*, vol. 3, no. 11, Article ID e3618, 2008.

[73] J. S. Jensen, C. S. Bradshaw, S. N. Tabrizi, C. K. Fairley, and R. Hamasuna, "Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance," *Clinical Infectious Diseases*, vol. 47, no. 12, pp. 1546–1553, 2008.

[74] R. Hamasuna, Y. Osada, and J. S. Jensen, "Antibiotic susceptibility testing of *Mycoplasma genitalium* by TaqMan 5′ nuclease real-time PCR," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 12, pp. 4993–4998, 2005.

[75] P. Judlin, Q. Liao, Z. Liu, P. Reimnitz, B. Hampel, and P. Arvis, "Efficacy and safety of moxifloxacin in uncomplicated pelvic inflammatory disease: the MONALISA study," *An International Journal of Obstetrics and Gynaecology*, vol. 117, no. 12, pp. 1475–1484, 2010.

[76] P. Barry, K. Ahrens, A. Roger et al., "Prevalence and incidence of *Mycoplasma genitalium* and *Trichomonas vaginalis* infections among STD clinic patients determined by transcription mediated amplification San Francisco and Denver, 2004/2005," *International Society for STD Research Book of Abstracts*, 2007.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 561909, 13 pages
doi:10.1155/2011/561909

Review Article

Treatment of Acute Pelvic Inflammatory Disease

Richard L. Sweet

Department of Obstetrics and Gynecology, University of California, Davis, CA 95817, USA

Correspondence should be addressed to Richard L. Sweet, richard.sweet@ucdmc.ucdavis.edu

Received 19 July 2011; Accepted 13 September 2011

Academic Editor: Thomas Cherpes

Pelvic inflammatory disease (PID), one of the most common infections in nonpregnant women of reproductive age, remains an important public health problem. It is associated with major long-term sequelae, including tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. In addition, treatment of acute PID and its complications incurs substantial health care costs. Prevention of these long-term sequelae is dependent upon development of treatment strategies based on knowledge of the microbiologic etiology of acute PID. It is well accepted that acute PID is a polymicrobic infection. The sexually transmitted organisms, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, are present in many cases, and microorganisms comprising the endogenous vaginal and cervical flora are frequently associated with PID. This includes anaerobic and facultative bacteria, similar to those associated with bacterial vaginosis. Genital tract mycoplasmas, most importantly *Mycoplasma genitalium*, have recently also been implicated as a cause of acute PID. As a consequence, treatment regimens for acute PID should provide broad spectrum coverage that is effective against these microorganisms.

1. Introduction

Pelvic inflammatory disease (PID) is a spectrum of upper genital tract infections that includes endometritis, salpingitis, tuboovarian abscess, and/or pelvic peritonitis [1]. Typically, acute PID is caused by ascending spread of microorganisms from the vagina and/or endocervix to the endometrium, fallopian tubes, and/or adjacent structures [1–3]. Acute salpingitis is the most important component of the PID spectrum because of its impact on future fertility [3].

PID is one of the most frequent and important infections that occur among nonpregnant women of reproductive age and remains a major public health problem [4–8]. Among women, it is the most significant complication of sexually transmitted diseases/infections. Unfortunately, women who acquire acute PID are at risk for long-term sequelae including tubal factor infertility, ectopic pregnancy, chronic pelvic pain, and recurrent PID [9–13]. In addition, the estimated annual health care cost for PID and its complications in the United States is over $2 billion [7].

Currently, an estimated 770,000 cases of acute PID are diagnosed annually in the United States. A recent analysis by the Centers for Disease Control and Prevention (CDC) of trends in the incidence of PID demonstrated that from 1985 to 2001 rates of both hospitalized and ambulatory cases of acute PID declined (68% and 47%, resp.) [6]. This good news is mitigated by two factors. Recently, subclinical PID has been recognized as an important entity which is common among women with lower genital tract infections, especially *Chlamydia trachomatis, Neisseria gonorrhoeae*, and bacterial vaginosis (BV) [14, 15]. Subclinical PID is as likely as clinically recognized acute PID and is responsible for a greater proportion of PID-related sequelae than clinically recognized disease [16]. Secondly, is concern that the continued increases in *C. trachomatis* infections reported by the CDC in the United States will be associated with an increase in both clinical and subclinical PID.

Over the past 25 years, important advances have occurred in understanding the etiology, pathogenesis, and treatment of acute PID. As a result, major paradigm shifts have occurred in our approach to the treatment of acute PID. In the past PID was believed to be a monoetiologic infection, primarily caused by *Neisseria gonorrhoeae*. Today, the polymicrobic etiology of PID is well established and has led to utilization of broad spectrum antimicrobial regimens for treatment of acute PID [1, 2, 17, 18].

2. Etiology of PID

Prevention of the significant long-term complications associated with PID requires development of effective treatment strategies. Such treatment regimens are dependent upon an understanding of the microbiologic etiology of acute PID. However, elucidation of the etiology of PID has been hindered by several factors. Firstly, most studies have utilized specimens obtained from the lower genital tract (primarily cervix) and not the upper genital tract (endometrial cavity, fallopian tubes) which is the actual site of infection. Secondly, most investigations primarily focused on the sexually transmitted pathogens *N. gonorrhoeae* and/or *C. trachomati,s* and few studies have assessed the role of non-STD pathogens, especially anaerobic bacteria. Thirdly, even fewer investigations have addressed the putative role of *Mycoplasma genitalium* in the etiology of PID.

PID results from the intracannicular ascending spread of microorganisms from the cervix and/or vagina into the upper genital tract. Prior to the mid-1970s, PID was believed to be a monoetiologic infection due primarily to *N. gonorrhoeae*. Based initially upon culdocentesis studies of peritoneal fluid (Figure 1) and subsequently studies utilizing laparoscopy and/or endometrial aspirations to obtain specimens from the upper genital tract (Table 1) came the recognition that the etiology of acute PID is polymicrobic with a wide variety of microorganisms involved [1, 2, 19–41]. Included among these are *N. gonorrhoeae, C. trachomatis*, genital tract mycoplasmas (particularly *M. genitalium)*, anaerobic and aerobic bacteria which comprise the endogenous vaginal flora (e.g., *Prevotella* species, black-pigmented Gram-negative anaerobic rods, *Peptostreptococci* sp., *Gardnerella vaginalis, Escherichia coli, Haemophilus influenzae*, and aerobic streptococci).

Investigations by our group conducted in the 1980s that utilized laparoscopy and/or endometrial aspirations to obtain upper genital tract specimens demonstrated that approximately two-thirds of acute PID cases were associated with *N. gonorrhoeae* and/or *C. trachomatis* (Figure 2). In nearly one-third only anaerobic and aerobic bacteria are recovered. In addition, half of the women with *N. gonorrhoeae* and/or *C. trachomatis* had concomitant anaerobic and/or aerobic bacteria recovered. More recently, in the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) study, the largest treatment trial of mild to moderate acute PID in the US, *N. gonorrhoeae* and *C. trachomatis* were recovered in less than one-third of patients [42].

Many of the nongonococcal, nonchlamydial microorganisms recovered from the upper genital tract in acute PID are similar to those associated with bacterial vaginosis (BV), a complex perturbation of the vaginal flora leading to loss of hydrogen peroxide producing lactobacillus and overgrowth of *G. vaginalis, Prevotella* sp. (especially *P. bivius, P. disiens*, and *P. capillosus*), *Mobiluncus* sp., black-pigmented anaerobic Gram-negative rods, alpha-hemolytic streptococci, and mycoplasmas [43]. Multiple investigations have demonstrated an association between BV and acute PID [31, 35, 43–51]. In addition, use of a broad-range 16SrDNA polymerase chain reaction to identify uncultivable bacteria has identified bacterial 16S sequences of anaerobic bacteria associated with BV in the fallopian tube of women with laparoscopically confirmed acute PID [52].

Although *M. genitalium* was identified in the early 1980s as a cause of nongonococcal urethritis in men, its role in genital tract infections in women remained unclear, due in large part to difficulty in culturing this organism. With the advent of polymerase chain reaction (PCR) technology, *M. genitalium* has been associated with cervicitis [53, 54] and has been demonstrated as an etiologic agent in nongonococcal nonchlamydial PID [36–39]. Haggerty et al. detected *M. genitalium* in 15% of women in the PEACH study [40], a rate similar to that seen in UK women (13%) [37] and west African women (16%) [36]. These rates of *M. genitalium* are similar to those seen for *C. trachomatis* and *N. gonorrhoeae* in the PEACH study of urban women in the United States. A recent analysis from the PEACH study noted that rates of short-term failure (persistent endometritis and pelvic pain), infertility, recurrent PID, and chronic pelvic pain were high among women with endometrial *M. genitalium* at baseline [40]. Subsequently, it has been demonstrated that women with *M. genitalium* infection (similar to those with chlamydial infection) present with fewer clinical signs and symptoms of acute PID than those with gonococcal infection [41]. A pathogenic role of *M. genitalium* in PID is further supported by studies demonstrating that *M. genitalium* induces salpingitis in experimental monkey studies [55] and adheres to human fallopian tube epithelial cells, in organ culture, causing damage to ciliated cells [56].

Recent attention has focused on subclinical PID. This term was initially applied to women with documented tubal factor infertility associated with evidence of chronic inflammatory residua characteristic of PID who denied a history of being diagnosed or treated for acute PID [15]. Preliminary work by our group has suggested that the microorganisms (e.g., *N. gonorrhoeae, C. trachomatis,* and bacterial vaginosis) associated with subclinical PID are the same putative agents recovered from women with clinically apparent acute PID [14].

3. Treatment Concepts

The therapeutic goals for treatment of acute PID include both short-term outcomes such as clinical cure and microbiologic cure and preventions of long-term sequelae such as infertility, ectopic pregnancy, recurrent infection, and chronic pelvic pain. Although the incidence rates of PID have declined, no reduction in the adverse reproductive outcomes associated with PID (infertility, ectopic pregnancy, and chronic pelvic pain) has been demonstrated [17].

While some antibiotic regimens have been successful in producing initial clinical and microbiologic cure with short-term followup, only a few studies have determined the efficacy of these treatment regimens for eliminating endometrial or fallopian tube infection. In addition, few studies have attempted to assess the incidence of long-term sequelae (e.g., tubal factor infertility, ectopic pregnancy and chronic pelvic

Table 1: Recovery of microorganisms from the upper genital tract of women with acute PID.

Study	Number of patients	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*	Anaerobic and aerobic bacteria
Sweet [26–29]	380	68 (18%)	172 (45%)	267 (70%)
Wasserheit [30]	23	11 (44%)	8 (35%)	11 (45%)
Heinonen [31]	25	10 (40%)	4 (16%)	17 (68%)
Paavonen [32]	35	12 (34%)	4 (11%)	24 (69%)
Brunham [33]	50	21 (42%)	8 (16%)	10 (20%)
Soper [34]	84[a]	1 (1.2%)	32 (38%)	12 (13%)
Hillier [35]	51[b]	6 (7.4%)	49 (98%)	16 (32%)
	85[a]	3 (4%)	16 (19%)	43 (50%)
	178[b]	23 (13%)	44 (25%)	168 (94%)
	278[c]	27 (9.9%)	37 (13.4%)	170 (61%)
Haggerty [36]	45[c,d]	12 (26.5%)	15 (33.3%)	[e]
Total	1234	194 (15.7%)	389 (31.5%)	770 (62%)

[a]Fallopian tube, cul-de-sac.
[b]Endometrial cavity.
[c]Clinically diagnosed acute PID.
[d]Histologic endometritis.
[e]Not available as total: anaerobic Gram-negative rods 31.7%; anaerobic Gram-positive cocci 22%; *Gardnerella vaginalis* 30.5%.
Reprinted with permission. Sweet [3].

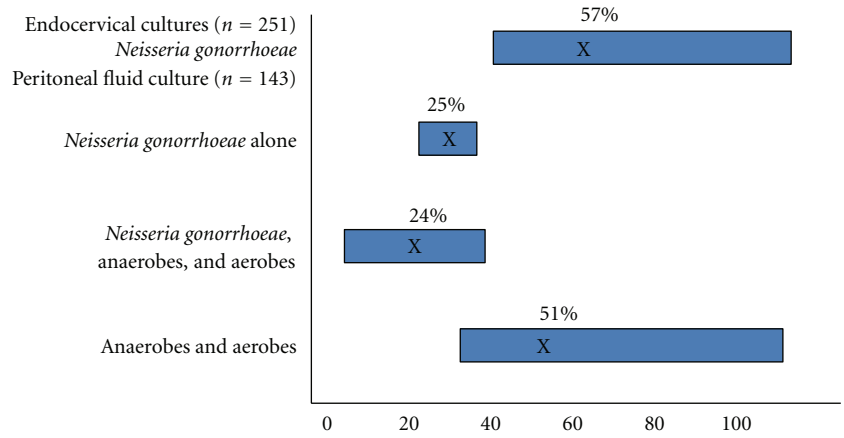


Figure 1: Microbiologic Etiology of Acute PID as determined by Culdocentesis, (based on references [20–25]).

pain) following treatment with these antibiotic regimens [1, 10, 11, 42].

In the preantibiotic era most cases of acute PID managed by conservative supportive care resolved spontaneously with studies demonstrating that approximately 85% of patients with acute PID improved clinically without the need for surgical intervention. The other 15% had prolonged or progressive symptoms requiring surgical intervention. In addition, there was approximately a 1% mortality rate. The introduction of antibiotics into clinical practice led to improvement in the prognosis for acute PID, and mortality was nearly eliminated. Studies assessing fertility rates following acute PID showed a general improvement in fertility with the mean pregnancy rate increasing from 27.9% (range 24%–43%) in the preantibiotic era to 73.1% (range 24%–81%) in the post-antibiotic era [57]. While this finding is satisfying, these results are still far from adequate.

As reviewed above, PID is a polymicrobial infection. According to the CDC, PID treatment regimens must provide broad spectrum coverage of likely pathogens [1]. Substantial evidence supports the role of *N. gonorrhoeae, C. trachomatis*, anaerobic bacteria, and facultative bacteria in the pathogenesis of acute PID [1–5, 9]. Not only are *N. gonorrhoeae* and *C. trachomatis* frequently recovered from the upper genital tract in women with PID, excellent data demonstrates the role these pathogens play in producing tubal damage and in the development of the adverse sequelae of PID (e.g., infertility, ectopic pregnancy) [57–60]. Thus, antimicrobial regimens for the treatment of acute PID must be effective against these STD organisms. While some

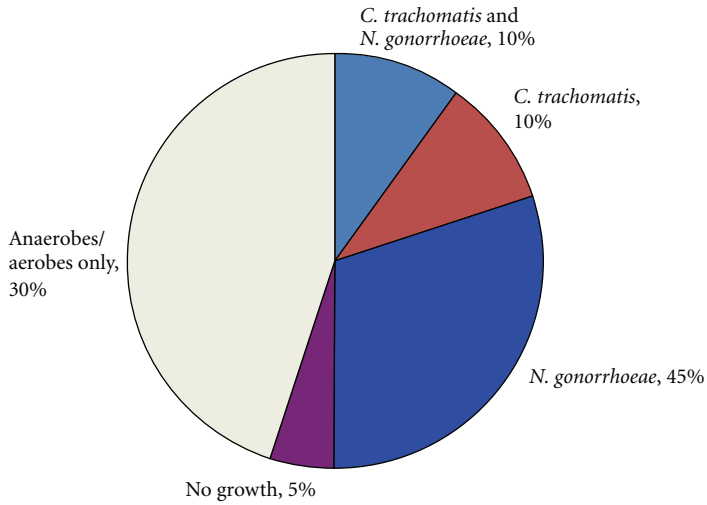


Figure 2: Microbiology of acute PID.

antimicrobial regimens that do not provide adequate coverage against *N. gonorrhoeae* and/or *C. trachomatis* have been shown to have excellent clinical cure rates, microbiologic cure rates are less impressive (or lacking), and long-term outcome data are not available [17, 18, 61–64]. The CDC in its 2010 treatment recommendations [1] notes that all regimens used to treat acute PID should provide adequate coverage against *N. gonorrhoeae* and *C. trachomatis*, as they are both commonly present and have the propensity to produce tubal damage directly (*N. gonorrhoeae*) or indirectly via the host immune response (*C. trachomatis*).

The putative role of nongonococcal nonchlamydial bacteria, especially anaerobes and more recently *M. genitalium*, in the pathogenesis of acute PID and whether antimicrobial regimens for treatment of PID should provide coverage against these microorganisms is more controversial. Some propose that anaerobic coverage is only required in patients with severe PID [2], especially those with tuboovarian abscesses. Others suggest that anaerobic coverage should be provided to all women with acute PID [1]. Clearly anaerobic bacteria have been demonstrated in the upper genital tract of women with acute PID with anaerobic bacteria recovered from the upper genital tract in 13% to 78% of women with PID [28–35]. In addition, anaerobes (e.g., *Bacteroides fragilis*) have caused tubal damage in vitro studies [1].

Bacterial vaginosis (BV) has been noted to be frequently present in women presenting with acute PID [1, 43, 51]. In the PEACH study, two-thirds of the women had concomitant BV [45]. Moreover, in the PEACH study women with acute endometritis on endometrial biopsy were commonly infected with BV-associated microorganisms in their upper genital tract (*G. vaginalis* 30.5%, anaerobic Gram-negative rods 31.7%, and anaerobic Gram-positive cocci 22%) [45]. Multiple previous studies [31, 43–49] support the findings of the PEACH study conclusions that BV is associated with acute PID. In addition, the Gyn Infectious Follow-through (GIFT) study, a longitudinal study of women with BV, demonstrated that the presence of BV-related microorganisms significantly increased the risk for acquiring PID [65].

The PEACH Study authors concluded that BV-associated organisms are very commonly present in women with mild-to-moderately severe PID and suggested that treatment regimens for all women with PID include antimicrobial agents effective against anaerobes associated with BV. In a similar vein, the CDC notes that until treatment regimens that do not adequately cover these BV-associated anaerobes have been demonstrated in clinical trial to prevent the long-term sequelae of PID as efficaciously as regimens which provide effective coverage for these microbes, use of regimens with antianaerobic activity should be considered.

Limited data suggest that failure to cover anaerobes in women with acute PID may predispose them to development of long-term sequelae. In the 1970s when single agent monotherapy was the standard for treatment of PID, Chow et al. noted that tuboovarian abscesses developed in PID patients being treated solely with tetracycline [19]. Subsequently, our group reported that anaerobic bacteria persisted in the endometrial cavities of women with PID treated with ciprofloxacin despite apparent clinical cure [62]. This finding is analogous to the finding by our group that failure to include an antimicrobial agent effective against *C. trachomatis* resulted in persistent chlamydial infection in the endometrial cavity [61]. In a proof of concept study, Eckert and coworkers demonstrated that women at high risk for PID but without a clinical diagnosis of PID improved with antimicrobial regimens that provided anaerobic coverage as measured by clinical improvement and resolution of histologic endometritis [66].

Neither the 2010 CDC sexually transmitted disease treatment guidelines [1] nor the 2007 European guideline for management of pelvic inflammatory disease [2] strongly advocate for anaerobic coverage in the treatment of acute PID. However, because of the substantial evidence that anaerobes are commonly recovered from women with mild-to-moderate and severe PID, and that failure to eradicate

anaerobes from the upper genital tract may lead to tubal damage, it seems prudent to do so. Firstly, as noted above, until those regimens that do not provide adequate anaerobic coverage have been shown to prevent adverse sequelae as well as those that do, it seems advisable to provide anaerobic coverage. A second strong reason for providing anaerobic coverage is the frequent (up to 70%) occurrence of BV in women with PID [50]. Thirdly, anaerobes are widely recognized as important pathogens in severe PID [67]. Severe PID, as determined by laparoscopy, not clinically, is an important determinant of future infertility [10, 68]. Thus, unless severe tubal disease has been excluded at laparoscopy, coverage for anaerobes may have important implications for the future reproductive health of these women.

On the other hand, reservation regarding the need for anaerobic coverage for acute PID has been raised. The PEACH trial [42] compared inpatient with outpatient treatment regimens in which patients were randomized to intravenous cefoxitin and doxycycline for a minimum of 48 hours (followed by oral doxycycline for a total of 14 days) or to a single dose of cefoxitin plus 2 weeks of oral doxycycline. In the ambulatory arm, the single dose of cefoxitin probably had little impact on anaerobic bacteria, whereas in the hospitalized arm patients received 48 hours of anaerobic therapy. No superiority was noted for either antimicrobial regimen, calling into question the need for anaerobic therapy in women with mild-to-moderate PID. In a recent editorial, Eschenbach also questioned a putative role for anaerobes in the pathogenesis of mild-to-moderate acute PID and suggested that although anaerobes may be present in the fallopian tubes, their role in the infectious process is not entirely clear [69].

However, concern remains about the importance of anaerobes in the pathogenesis and treatment of acute PID. Failing to provide anaerobic coverage in PID treatment regimen is problematic because there is limited data in support of the efficacy of such an approach. Hopefully, additional studies will address this issue and provide further insight into the role of anaerobes in PID.

Although recent reviews of PID treatment trials have noted that most antibiotic regimens, with the exception of the doxycycline and metronidazole regimen, result in fairly similar excellent clinical and microbiologic (primarily cervical *N. gonorrhoeae* and *C. trachomatis*) cure rates [17, 18, 63, 64], the search continues for treatment regimen(s) that optimize prevention of infertility, ectopic pregnancy, chronic pelvic pain, and recurrent infection. Three major determinants for preservation of post-PID fertility have been identified [3, 69]. These are (1) short duration of symptoms (<72 hours) prior to institution of therapy; (2) repetitive episodes of PID; (3) nongonococcal PID [16, 70, 71].

Duration of symptoms is the major determinant of subsequent infertility. Early diagnosis and treatment are crucial for preserving fertility and the effectiveness of antibiotic therapy is dependent upon the interval from the onset of symptoms to the initiation of treatment. In an updated analysis of the Lund, Sweden cohort of women with laparoscopically confirmed PID, Hillis and colleagues [71] demonstrated that women treated with ≥3 days of symptoms had a significantly greater infertility rate compared to those <3 days from symptom onset (19.7% versus 8.3%).

In their cohort of laparoscopically confirmed cases of PID, Westrom and colleagues reported that reinfection was an important predictor of subsequent tubal factor infertility [10]. In the most recent update of this cohort with 1,309 PID cases and 451 control patients who attempted to conceive, noted that the rate of infertility is directly proportional to both the number of episodes and severity of tubal inflammation seen at laparoscopy [11]. Each episode roughly doubles the rate of infertility; with one, two, or three or more episodes of PID infertility rates were 8.0%, 19.5%, and 40%, respectively. Among women with a single episode of PID, future fertility was associated with the severity of PID (at laparoscopy) ranging from 0.6% with mild disease to 6.2% and 21.4% for moderate and severe PID, respectively.

Studies based on the Swedish cohort [16, 70] have also demonstrated that women with chlamydial PID and nongonococcal nonchlamydial PID fared more poorly after treatment than those with gonococcal PID. Most likely for chlamydial PID, it is the delayed commencement of treatment associated with mild slow onset of symptoms. Nongonococcal nonchlamydial PID is more often associated with severe PID which is associated with a worse prognosis for future fertility.

4. Antimicrobial Treatment Regimens

Despite the controversy regarding the role of anaerobic bacteria and *M. genitalium* in the pathogenesis of acute PID, the polymicrobic nature of PID is widely acknowledged [1, 2]. As a consequence, PID is treated with antibiotics which provide coverage against a broad spectrum of potential pathogens. In 2010 the Center for Disease Control and Prevention updated their Guidelines for treatment of acute PID (Tables 2 and 3). According to the CDC 2010 guidelines, PID treatment regimens must provide empiric, broad spectrum coverage of likely pathogens [1]. These guidelines recommend that all treatment regimens should be effective against *N. gonorrhoeae* and *C. trachomatis* even in the presence of negative endocervical screening for these organisms. Although the CDC notes that the need to eradicate anaerobes from women with PID has not been definitively determined, as reviewed above, they suggest that until regimens without adequate coverage for anaerobes have been shown to prevent long-term sequelae as successfully as those that include anaerobic coverage, coverage of anaerobes should be considered in the treatment of acute PID.

As noted by the CDC [1] multiple randomized clinical treatment trials have demonstrated efficacy of both parenteral and oral regimens. In Table 4, the short-term clinical and microbiologic efficacy of oral and parenteral treatments regimens for PID are summarized. After excluding the metronidazole-doxycycline regimen (clinical and microbiologic cure rates 75% and 71%, resp.), the pooled clinical cure rates ranged from 88% to 99%, and the pooled microbiologic cure rates ranged from 89% to 100%. It is important that empiric treatment be initiated as soon as a presumptive

Table 2: Parenteral treatment recommendations for acute pelvic inflammatory disease[a].

Recommended regimen A
Cefotetan 2 g IV every 12 hours
Or
Cefoxitin 2 g IV every 6 hours
Plus
Doxycycline 100 mg orally or IV every 12 hours
Recommended regimen B
Clindamycin 900 mg IV every 8 hours
Plus
Gentamicin loading dose IV or IM (2 mg/Kg body weight) followed by a maintenance dose (1–5 mg/Kg body weight) every 8 hours.
A single daily dosing (3–5 mg/Kg) can be substituted
Alternative parenteral regimen
Ampicillin/sulbactam 3 g IV every 6 hours
Plus
Doxycycline 100 mg orally or IV every 12 hours

[a]CDC Sexually Transmitted Diseases Treatment Guidelines 2010 MMWR 2010 : 59 (no.-RR12): [63–67].

Table 3: Oral treatment recommendations for acute pelvic inflammatory disease[a].

Recommended regimens
(1) Ceftriaxone 250 mg IM in a single dose
Plus
Doxycycline 100 mg orally twice a day for 10–14 days
With or without
Metronidazole 500 mg orally twice a day for 10–14 days
(2) Cefoxitin 2 g IM in a single dose and Probenecid 1 g orally administered concomitantly as a single dose
Plus
Doxycycline 100 mg orally twice a day for 10–14 days
With or without
Metronidazole 500 mg orally twice a day for 10–14 days
(3) Other parenteral third generation cephalosporins (e.g., ceftizoxime or cefotaxime) in a single dose
Plus
Doxycycline 100 mg orally twice a day for 10–14 days
With or without
Metronidazole 500 mg orally twice a day for 10–14 days

[a]CDC Sexually Transmitted Diseases Treatment Guidelines 2010 MMWR 2010 : 59 (no.-RR12).

diagnosis of acute PID is made because prevention of long-term sequelae is determined to a large extent by early administration (<72 hours) of appropriate antimicrobial therapy [1]. In addition, selection of a treatment regimen should consider availability, cost, patient acceptance, and antimicrobial acceptability [1, 72].

Because parenteral antibiotics do not necessarily require hospitalization, antibiotic regimens for the treatment of acute PID are categorized as follows:

(1) regimens requiring more than a single parenteral dose as initial therapy are "parenteral" and

(2) regimens that are primarily oral with or without an initial single parenteral dose are considered "oral."

4.1. Parenteral Treatment. As noted in Table 4, several parenteral antimicrobial regimens have excellent short-term clinical and microbiological efficacy. Most of the literature supports the combination of (1) cefoxitin or cefotetan plus doxycycline and (2) clindamycin plus gentamicin. These two regimens remain the parenteral regimens recommended by the CDC for the treatment of PID. However, cefotetan is not currently marketed in the United States.

According to the CDC, there is limited data available supporting a role of other second or third generation parenteral cephalosporins (e.g., ceftizoxime, cefotaxime, or ceftriaxone) as effective therapy for acute PID and/or replacements for cefotetan or cefoxitin [1]. Moreover, these antimicrobial agents are less active against anaerobic bacteria than cefoxitin or cefotetan.

Intravenous infusion of doxycycline frequently causes pain and, thus, doxycycline should be administered orally whenever possible. Fortunately, oral and intravenous administration of doxycycline provide similar bioavailability [1].

With parenteral regimen A, parenteral therapy can be discontinued 24 hours after clinical improvement occurs [1]. However, oral doxycycline (100 mg twice a day) should be continued to complete a 14-day course of therapy. In cases involving tuboovarian abscess, either clindamycin (450 mg orally four times a day) or metronidazole (500 mg orally every 6 hours) should be used for continued therapy in order to provide more effective coverage against anaerobic bacteria.

There is concern over the increasing resistance of anaerobes, especially the *Bacteroides fragilis* group, to clindamycin [73, 74]. However, based on multiple clinical studies and extensive successful results with clindamycin containing regimens, clindamycin remains as a component in one of the recommended parenteral treatment regimens in both the CDC [1] and European [2] guidelines for treatment of PID.

Single dose gentamicin has not been evaluated for the treatment of acute PID. However, it is efficacious in the treatment of other pelvic and abdominal infections and is an option in parenteral regimen B. With this regimen, parenteral therapy may be discontinued 24 hours after clinical therapy. While the CDC suggests that either doxycycline 100 mg orally twice a day or clindamycin 450 mg orally four times a day to complete a total of 14 days of therapy may be used [1], in the author's opinion clindamycin oral therapy is preferred because of its better anaerobic coverage. In the presence of severe PID, especially tuboovarian abscess, clindamycin continued therapy is recommended by the CDC [1].

Table 4: Clinical and microbiologic cure rates for pelvic inflammatory disease treatment regimens.

Regimen	Clinical cure			Microbiologic cure		
	Number of studies	Number of patients	Percent	Number of studies	Number of patients	Percent
Parenteral						
Clindamycin/aminoglycoside	11	470	92	8	143	97
Cefoxitin/doxycycline	9	836	95	7	581	96
Cefotetan/doxycycline	3	174	94	2	71	100
Ciprofloxacin	4	90	94	4	72	96
Ofloxacin	2	86	99	2	50	98
Sulbactam-ampicillin/doxycycline	1	37	95	1	33	100
Metronidazole/doxycycline	2	36	75	1	7	71
Azithromycin	1	30	100	1	30	100
Azithromycin/metronidazole	1	30	97	1	30	97
Oral						
Ceftriaxone/probenecid/doxycycline	1	64	95	1	8	100
Cefoxitin/probenecid/doxycycline	3	212	90	3	71	93
Cefoxitin/doxycycline	4	634	94	4	493	95
Amoxicillin-clavulanic acid	2	35	100	2	35+	100
Ciprofloxacin/clindamycin	1	67	97	1	10	90
Ofloxacin	2	165	95	2	42+	100
Levofloxacin	1	41	85	1	9	89

Reprinted with permission from Walker and Sweet [64].

As noted by Walker and Wiesenfeld [17], there has been renewed interest in alternative agents, particularly ampicillin-sulbactam for anaerobic coverage. Unlike clindamycin, this agent has not been associated with selective pressure for microbial resistance. In addition, ampicillin-sulbactam is effective for *N. gonorrhoeae*. To provide adequate coverage for *C. trachomatis*, concomitant administration of doxycycline is recommended. Following clinical improvement, oral therapy with doxycycline 100 mg twice a day to complete 14 days of therapy should be continued. With severe disease, especially TOA, metronidazole 500 mg orally four times daily should be commenced as well.

While not included in the CDC 2010 recommended or alternative regimens for the treatment of PID, several factors have led clinicians to use azithromycin for the treatment of acute PID. These include widespread use in treating chlamydial infection, enhanced compliance due to its long half-life, and studies demonstrating the anti-inflammatory effects of macrolide antibiotics including azithromycin which appear to enhance host defense mechanisms and restrict local inflammation [17, 18, 75, 76]. A randomized clinical trial in the United Kingdom among 300 women with laparoscopically confirmed PID demonstrated excellent short-term clinical care rates with azithromycin monotherapy for one week (500 mg IV daily for one or two days followed by 250 mg for 5-6 days) or in combination with a 12 day course of metronidazole [77]. The microbiologic cure rates were also excellent (>95%) for *N. gonorrhoeae, C. trachomatis, M. hominis,* and anaerobes with these regimens. However, there was a large dropout rate with only one-third of the patients completing the study per protocol which, as noted by Haggerty and Ness [18] reduced the validity and generalizability of the microbiological cure evaluation. In addition, the anaerobic bacteria were only recovered from 27 (9%) of the patients, a rate substantially lower than noted in other studies.

The 2007 European guideline for the management of pelvic inflammatory disease contains similar recommendations [2].

As alternative regimens, the European guideline suggests i.v. ofloxacin 400 mg twice daily plus i.v. metronidazole 500 mg three times daily for 14 days or i.v. ciprofloxacin 200 mg twice daily plus i.v. (or oral) doxycycline 100 mg twice daily plus i.v. metronidazole 500 mg three times daily [2]. Because anaerobes are probably of relatively greater importance in patients with severe PID and some studies have demonstrated good clinical response without the use of metronidazole, the European guideline suggests that metronidazole may be discontinued in those patients with mild or moderate PID who are unable to tolerate it [2]. They further note that ofloxacin and moxifloxacin should be avoided in patients who are at high risk of gonococcal PID due to increasing quinolone resistance by *N. gonorrhoeae.*

4.2. Oral Treatment. Over the past 20 years, a new paradigm has emerged with a dramatic shift from hospital-based parenteral antibiotic regimens to oral ambulatory-based regimens [6, 7]. Initially, this shift was largely driven by the emergence of managed care and other economic factors without the benefit of clinical studies demonstrating that oral

therapy was as effective as parenteral regimens, especially for prevention of long-term sequelae.

The PEACH study has provided evidence supporting the use of oral regimens on an ambulatory basis for the treatment of mild and moderately severe acute PID [42, 78]. PEACH, the largest randomized clinical treatment trial of acute PID in the United States, compared inpatient parenteral therapy (intravenous cefoxitin and oral or intravenous doxycycline during ≥48-hour hospitalization followed by oral doxycycline to complete a 14 day course) with outpatient oral therapy (a single intramuscular dose of cefoxitin with doxycycline administration orally for 14-days). Of most significance, PEACH not only assessed short-term but also long-term outcomes for over 800 patients (398 inpatient and 410 outpatient) with mild-to-moderately severe PID. The short-term clinical cure rates at 30 days were excellent in both groups, with roughly 3% of women in each group requiring additional treatment. At a mean followup of 35 months, the pregnancy rates were 42.0% and 41.7% with the outpatient and inpatient regimens, respectively. Long-term outcomes including infertility, ectopic pregnancy, recurrent PID, and chronic pelvic pain were also similar in both groups. However, as emphasized by Haggerty and Ness, despite high rates of clinical cure and eradication of *N. gonorrhoeae* and *C. trachomatis*, the rates of infertility (17%), recurrent PID (14%), and chronic pelvic pain (37%) were disappointingly high [18].

While data from the PEACH study suggests that neither the route nor site of treatment administration affects short-term or long-term outcomes among women with mild-to-moderately severe acute PID [42, 78], higher rates of post treatment histologic endometritis were present among the women in the outpatient oral group. However, the clinical significance of this finding is not clear. In previous studies we have shown that ongoing subclinical PID (as defined by histologic acute endometritis) is frequently present in women with untreated lower genital tract infections [14], and that persistent endometrial infection with *C. trachomatis* [26] and anaerobes [22] may lead to subsequent tubal damage and increased infertility among women with inadequately treated acute PID. Similarly, among women enrolled in the PEACH study, 23 out of 56 (41%) with *M. genitalium* identified in either the cervix and/or endometrium at baseline had *M. genitalium* persistently identified 30 days following treatment (inadequate to treat this organism) [40]. Moreover, women with persistent endometrial *M. genitalium* were 4.5 times more likely to experience short-term treatment failure (i.e., histologic endometritis and persistent pelvic pain at the 30-day follow-up visit).

As noted by the CDC [1], outpatient oral therapy can be considered for treatment of women with mild-to-moderately severe acute PID. The oral regimens listed in Table 3 provide coverage against the major etiologic agents of acute PID. Which of the cephalosporins is the optimum selection is unclear [1]. On the one hand cefoxitin has better anaerobic coverage, while ceftriaxone has better coverage against *N. gonorrhoeae*. The dose of ceftriaxone was increased to 250 mg IM in the 2010 CDC guidelines [1]. The extent of efficacy against anaerobic bacteria with a single dose of cefoxitin is questionable. However, in the PEACH study single dose cefoxitin was effective in obtaining clinical response [42, 78]. The CDC [1] and Walker and Wiesenfeld [17] have noted that theoretical limitations in coverage of anaerobes by recommended cephalosporins may require addition of metronidazole to the oral treatment recommendations. Addition of metronidazole to the oral regimens is the author's preferred approach. In addition, metronidazole will effectively treat bacterial vaginosis, which as noted above is frequently associated with PID. There is no published data on the use of oral cephalosporins for treatment of acute PID [1].

Information regarding alternative oral (outpatient) regimens is quite limited. Several alternative regimens have been the subject of at least one clinical trial and contain broad spectrum coverage [1]. These include (1) amoxicillin/clavulanic acid and doxycycline [79] and (2) Azithromycin monotherapy [77] or a combination of ceftriaxone 250 mg IM single dose with azithromycin 1 gram orally once a week for two weeks [80]. If one of these alternative regimens is selected, the CDC suggests the addition of metronidazole should be considered to cover anaerobic bacteria which are suspected as etiologic agents in PID and to effectively treat concomitant BV [1].

With the emergence of quinolone-resistant *N. gonorrhoeae*, regimens that include a quinolone agent are no longer recommended by the CDC for treatment of acute PID [1]. They note that in situations where single dose parenteral cephalosporin is not feasible, use of fluoroquinolones (levofloxacin 500 mg orally once a day or ofloxacin 400 mg orally twice a day for 14 days) with or without metronidazole (500 mg twice daily for 14 days) can be considered if community prevalence and individual risk for gonorrhea are low [1]. If this approach is selected, the CDC stresses that diagnostic tests for *N. gonorrhoeae* must be performed prior to initiating treatment [1]. Culture is the preferred test. If *N. gonorrhoeae* is detected, treatment should be based on the results of antimicrobial susceptibility. With quinolone-resistant *N. gonorrhoeae* or if susceptibility cannot be assessed (e.g., nucleic acid amplification test) use of a parenteral cephalosporin is recommended [1]. If use of a cephalosporin is not feasible, azithromycin 2 grams as a single dose can be added to a quinolone-based PID treatment regimen [1].

Patients treated with an oral regimen should demonstrate substantial clinical improvement within three days following initiation of treatment [1]. Clinical improvement is determined by defervescence, reduction in direct or rebound abdominal tenderness, and/or reduction in uterine, adnexal, and cervical motion tenderness. When patients fail to improve within this window, hospitalization is usually required for additional diagnostic tests (e.g., rule out TOA), parenteral antibiotic therapy, and/or surgical intervention [1].

4.3. Hospitalization for Treatment of Acute PID. While in the past, and to a lesser extent today, some clinicians have recommended that all patients with PID be hospitalized for parenteral antibiotics and bed rest, the PEACH study clearly

Table 5: Suggested criteria for hospitalization for treatment of acute PID[a].

(i) Surgical emergencies (e.g, appendicitis) cannot be ruled out
(ii) Patient is pregnant
(iii) Patient does not respond clinically to oral antimicrobial therapy
(iv) Patient unable to follow or tolerate outpatient oral regimen
(v) Patient has severe illness, nausea, vomiting, or high fever
(vi) Patient has a tuboovarian abscess

[a]CDC Sexually Transmitted Diseases Treatment Guidelines 2010 MMWR 2010 : 59 (no.-RR12).

demonstrated that in women with mild-to-moderately severe PID, outpatient oral therapy results in similar short- and long-term clinical outcomes as inpatient therapy [42]. As a result, the CDC notes that a decision regarding the need for hospitalization should be based on the judgment of the health-care provider and whether the patient meets any of the CDC suggested criteria for hospitalizations (Table 5). The European guideline concurs with these recommendations [2].

Limited studies have demonstrated that pregnant women with PID have high rates of fetal wastage and preterm delivery, supporting the appropriateness of hospitalization [81, 82]. Similarly, ample data supports hospitalization of women with TOAs in order to maximize antimicrobial dosing and close monitoring for early recognition of severe sepsis or of leaking/rupture of the abscess.

Several previous criteria for hospitalization have been removed from the current suggestions. The absence of data to support benefit from hospitalization for adolescent girls with PID led the CDC to not list adolescence among the criteria for hospitalization and to suggest that a decision to hospitalize adolescents with PID should be based on the same criteria used for older women [1]. In fact, subanalysis of the outcome data of the PEACH study stratified by age demonstrated that fertility outcomes of the adolescents were similar in the inpatient and outpatient treatment arms [78]. However, some clinicians continue to advocate that all adolescents and never pregnant young women should be hospitalized for treatment [83]. They argue that adolescence is a proxy for poor compliance, high-risk sexual activity, delayed care, and high antimicrobial failure rates.

Whereas the presence of HIV infection or immunosuppression has previously been an indicator for hospitalization and parenteral therapy, currently it is recommended that HIV-positive women with acute PID can be treated similarly to HIV-negative women. Although HIV-infected women who develop PID may have more severe clinical presentations and are more likely to have TOAs [84–86], there is no evidence to suggest that immunocompromised women benefit from hospitalization or parenteral therapy for uncomplicated PID [17, 87, 88].

4.4. Management of PID Associated with Intrauterine Contraceptive Device (IUD). With the renewed interest in the IUD as a contraceptive choice for young women, PID will be seen in women using IUDs. As noted by Walker and Wiesenfeld, there does not exist any data to indicate that selection of treatment regimens should be influenced by the presence of an IUD [17]. In the past, clinicians generally removed IUDs to optimize the treatment of PID. This was primarily based on concerns that as a foreign body, removal of the IUD enhanced clinical response. Only a few studies have addressed this issue and the results are conflicting. In a small randomized study of 46 women in Sweden, Soderberg, and Lindgren [89] reported no differences in response to treatment whether the IUD was removed or left in place. On the other hand, Altunyurt and colleagues, in a randomized trial from Turkey, noted that clinical improvement (e.g., absence of pelvic pain, vaginal discharge, and pelvic tenderness) was more common in the group whose IUDs were removed [90]. If the provider elects to leave the IUD in place while PID is being treated, close clinical followup is important.

4.5. Management of Sex Partners. According to the CDC, male sex partners of women diagnosed with acute PID should be examined and treated if they had sexual contact with the patient during the preceding 60 days. If the last episode of sexual intercourse was > 60 days prior to onset of symptoms, the last sexual partner should be treated [1]. Women diagnosed with acute PID should refrain from sexual intercourse until treatment is completed and they and their partner(s) are asymptomatic. Sex partners of women with PID should be treated empirically with regimens effective against *N. gonorrhoeae* and *C. trachomatis* [1]. In those settings where only women are treated, arrangements should be undertaken to either provide care or appropriate referral for male sex partners [1]. Expedited partner treatment or enhanced patient referral are acceptable alternative approaches for the treatment of male partners of women who have PID with chlamydial or gonococcal infection [1].

5. Conclusion

Treatment strategies for women with acute PID should be based on the polymicrobial nature of this infection. The microorganisms recovered from the upper genital tract of women with acute PID include *N. gonorrhoeae, C. trachomatis*, and anaerobic and aerobic bacteria common to the endogenous vaginal flora and genital mycoplasmas, especially *M. genitalium*. Several antibiotic regimens are available which meet these requirements. Several parenteral antimicrobial regimens have been shown to provide very good short-term clinical and microbiological efficacy; these include clindamycin plus gentamicin, cefoxitin plus doxycycline, and cefotetan plus doxycycline.

Oral therapy for acute PID is currently the most commonly used approach, in response to both economic issues and the evidence from the PEACH study demonstrating that both short-term and long-term outcomes were similar for the oral and parenteral regimens. Due to the increased quinolone resistance of *N. gonorrhoeae*, choices of oral regimens are more limited. Ceftriaxone or cefoxitin demonstrated excellent short-term clinical and microbiological results. The addition of oral metronidazole to this regimen

is suggested by some experts including this author to provide improved anaerobic coverage and at least to treat BV which is present in up to 70% of women with acute PID.

Currently regimens recommended by the CDC for the treatment of acute PID provide suboptimal antimicrobial activity against *M. genitalium* [40]. Mycoplasma lack a cell wall and thus are resistant to beta-lactam antibiotics (e.g., cefoxitin, cefotetan, ceftriaxone). Increased tetracycline resistance among *M. genitalium* has been reported [91]. In addition, *M. genitalium* is associated with persistent nongonococcal urethritis treated with tetracyclines [92]. Variable resistance to fluoroquinolones has been reported [93]. Recently, a newer fluoroquinolone, moxifloxacin, has demonstrated excellent activity against *M. genitalium* [91, 93]. This agent is one of the outpatient regimens recommended in the European guidelines [2]. While *M. genitalium* has demonstrated susceptibility to macrolides, azithromycin resistance has recently been reported [94].

References

[1] K. A. Workowski and S. Berman, "Sexually transmitted diseases treatment guidelines," *Morbidity and Mortality Weekly Report*, vol. 59, no. 12 RR, pp. 63–67, 2010.

[2] J. Ross, P. Judlin, and L. Nilas, "European guideline for the management of pelvic inflammatory disease," *International Journal of STD and AIDS*, vol. 18, no. 10, pp. 662–666, 2007.

[3] R. L. Sweet, "Microbiology," in *Pelvic Inflammatory Disease*, R. L. Sweet and H. C. Wiesenfeld, Eds., pp. 19–48, Taylor & Francis, London, UK, 2006.

[4] C. L. Haggerty and R. B. Ness, "Diagnosis and treatment of pelvic inflammatory disease," *Women's Health*, vol. 4, no. 4, pp. 383–397, 2008.

[5] K. J. Smith, R. B. Ness, H. C. Wiesenfeld, and M. S. Roberts, "Cost-effectiveness of alternative outpatient pelvic inflammatory disease treatment strategies," *Sexually Transmitted Diseases*, vol. 34, no. 12, pp. 960–966, 2007.

[6] M. Y. Sutton, M. Sternberg, A. Zaidi, M. E. S. Louis, and L. E. Markowitz, "Trends in pelvic inflammatory disease hospital discharges and ambulatory visits, United States, 1985-2001," *Sexually Transmitted Diseases*, vol. 32, no. 12, pp. 778–784, 2005.

[7] D. B. Rein, W. J. Kassler, K. L. Irwin, and L. Rabiee, "Direct medical cost of pelvic inflammatory disease and its sequelae: decreasing, but still substantial," *Obstetrics and Gynecology*, vol. 95, no. 3, pp. 397–402, 2000.

[8] J. Botte and J. F. Peipert, "Epidemiology," in *Pelvic Inflammatory Disease*, R. L. Sweet and H. C. Wiesenfeld, Eds., pp. 1–18, Taylor & Francis, London, UK, 2006.

[9] C. L. Haggerty and R. B. Ness, "Epidemiology, pathogenesis and treatment of pelvic inflammatory disease," *Expert Review of Anti-Infective Therapy*, vol. 4, no. 2, pp. 235–247, 2006.

[10] L. Westrom, "Effect of acute pelvic inflammatory disease on fertility," *American Journal of Obstetrics and Gynecology*, vol. 121, no. 5, pp. 707–713, 1975.

[11] L. Westrom, R. Joesoef, G. Reynolds, A. Hagdu, and S. E. Thompson, "Pelvic inflammatory disease and fertility: a cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results," *Sexually Transmitted Diseases*, vol. 19, no. 4, pp. 185–192, 1992.

[12] C. L. Haggerty, R. Schulz, and R. B. Ness, "Lower quality of life among women with chronic pelvic pain after pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 102, no. 5, pp. 934–939, 2003.

[13] A. R. Thurman and D. E. Soper, "Sequelae," in *Pelvic Inflammatory Disease*, R. L. Sweet and H. C. Wiesenfeld, Eds., pp. 69–84, Taylor & Francis, London, UK, 2006.

[14] H. C. Wiesenfeld, S. L. Hillier, M. A. Krohn et al., "Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 100, no. 3, pp. 456–463, 2002.

[15] P. Wolner-Hanssen, N. B. Kiviat, and K. K. Holmes, "Atypical pelvic inflammatory disease: subacute, chronic or subclinical upper genital tract infection in women," in *Sexually Transmitted Diseases*, K. K. Holmes, P.-A. Mardh, and P. F. Sparling, Eds., pp. 615–620, McGraw–Hill, New York, NY, USA, 1990.

[16] L. Westrom and D. A. Eschenbach, "Pelvic inflammatory disease," in *Sexually Transmitted Diseases*, K. K. Holmes, P. F. Sparling, P.-A. Mardh et al., Eds., pp. 783–809, McGraw–Hill, New York, NY, USA, 1999.

[17] C. K. Walker and H. C. Wiesenfeld, "Antibiotic therapy for acute pelvic inflammatory disease: the 2006 centers for disease control and prevention sexually transmitted diseases treatment guidelines," *Clinical Infectious Diseases*, vol. 44, no. 3, pp. S111–S122, 2007.

[18] C. L. Haggerty and R. B. Ness, "Newest approaches to treatment of pelvic inflammatory disease: a review of recent randomized clinical trials," *Clinical Infectious Diseases*, vol. 44, no. 7, pp. 953–960, 2007.

[19] A. W. Chow, K. L. Malkasian, J. R Marshall et al., "The bacteriology of acute pelvic inflammatory disease. Value of cul de sac cultures and relative importance of gonococci and other aerobic or anaerobic bacteria," *American Journal of Obstetrics and Gynecology*, vol. 122, no. 7, pp. 876–879, 1975.

[20] D. A. Eschenbach, T. M. Buchanan, and H. M. Pollock, "Polymicrobial etiology of acute pelvic inflammatory disease," *New England Journal of Medicine*, vol. 293, no. 4, pp. 166–171, 1975.

[21] G. R. G. Monif, S. L. Welkos, H. Baer, and R. J. Thompson, "Cul de sac isolates from patients with endometritis salpingitis peritonitis and gonococcal endocervicitis," *American Journal of Obstetrics and Gynecology*, vol. 126, no. 2, pp. 158–161, 1976.

[22] F. G. Cunningham, J. C. Hauth, and L. C. Gilstrap, "The bacterial pathogenesis of acute pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 52, no. 2, pp. 161–164, 1978.

[23] R. L. Sweet, D. L. Draper, and J. Schachter, "Microbiology and pathogenesis of acute salpingitis as determined by laparoscopy: what is the appropriate site to sample?" *American Journal of Obstetrics and Gynecology*, vol. 138, no. 7, pp. 985–989, 1980.

[24] S. E. Thompson 3rd, W. D. Hager, and K. H. Wong, "The microbiology and therapy of acute pelvic inflammatory disease in hospitalized patients," *American Journal of Obstetrics and Gynecology*, vol. 136, no. 2, pp. 179–186, 1980.

[25] R. L. Sweet, "Sexually transmitted diseases. Pelvic inflammatory disease and infertility in women," *Infectious Disease Clinics of North America*, vol. 1, no. 1, pp. 199–215, 1987.

[26] R. L. Sweet, J. Schachter, and M. O. Robbie, "Failure of β-lactam antibiotics to eradicate *Chlamydia trachomatis* in the endometrium despite apparent clinical cure of acute salpingitis," *Journal of the American Medical Association*, vol. 250, no. 19, pp. 2641–2645, 1983.

[27] R. L. Sweet, J. Schachter, D. V. Landers, M. Ohm-Smith, and M. O. Robbie, "Treatment of hopitalized patients with acute

pelvic inflammatory disease: comparison of cefotetan plus doxycycline and cefoxitin plus doxycycline," *American Journal of Obstetrics and Gynecology*, vol. 158, no. 3, supplement, pp. 736–743, 1988.

[28] D. V. Landers, P. Wolner-Hanssen, J. Paavonen et al., "Combination antimicrobial therapy in the treatment of acute pelvic inflammatory disease," *American Journal of Obstetrics and Gynecology*, vol. 164, no. 3, pp. 849–858, 1991.

[29] J. N. Wasserheit, T. A. Bell, and N. B. Kiviat, "Microbial causes of proven pelvic inflammatory disease and efficacy of clindamycin and tobramycin," *Annals of Internal Medicine*, vol. 104, no. 2, pp. 187–193, 1986.

[30] P. K. Heinonen, K. Teisala, and R. Punnonen, "Anatomic sites of upper genital tract infection," *Obstetrics and Gynecology*, vol. 66, no. 3, pp. 384–390, 1985.

[31] J. Paavonen, K. Teisala, and P. K. Heinonen, "Microbiological and histopathological findings in acute pelvic inflammatory disease," *British Journal of Obstetrics and Gynaecology*, vol. 94, no. 5, pp. 454–460, 1987.

[32] R. C. Brunham, B. Binns, F. Guijon et al., "Etiology and outcome of acute pelvic inflammatory disease," *Journal of Infectious Diseases*, vol. 158, no. 3, pp. 510–517, 1988.

[33] D. E. Soper, N. J. Brockwell, H. P. Dalton, and D. Johnson, "Observations concerning the microbial etiology of acute salpingitis," *American Journal of Obstetrics and Gynecology*, vol. 170, no. 4, pp. 1008–1017, 1994.

[34] S. L. Hillier, N. B. Kiviat, S. E. Hawes et al., "Role of bacterial vaginosis-associated microorganisms in endometritis," *American Journal of Obstetrics and Gynecology*, vol. 175, no. 2, pp. 435–441, 1996.

[35] C. L. Haggerty, S. L. Hillier, D. C. Bass, and R. B. Ness, "Bacterial vaginosis and anaerobic bacteria are associated with endometritis," *Clinical Infectious Diseases*, vol. 39, no. 7, pp. 990–995, 2004.

[36] C. R. Cohen, L. E. Manhart, E. A. Bukusi et al., "Association between *Mycoplasma genitalium* and acute endometritis," *The Lancet*, vol. 359, no. 9308, pp. 765–766, 2002.

[37] I. Simms, K. Eastick, H. Mallinson et al., "Associations between *Mycoplasma genitalium*, *Chlamydia trachomatis* and pelvic inflammatory disease," *Journal of Clinical Pathology*, vol. 56, no. 8, pp. 616–618, 2003.

[38] C. R. Cohen, N. R. Mugo, S. G. Astete et al., "Detection of *Mycoplasma genitalium* in women with laparoscopically diagnosed acute salpingitis," *Sexually Transmitted Infections*, vol. 81, no. 6, pp. 463–466, 2005.

[39] H. F. Svenstrup, J. Fedder, S. E. Kristoffersen, B. Trolle, S. Birkelund, and G. Christiansen, "*Mycoplasma genitalium*, *Chlamydia trachomatis*, and tubal factor infertility-a prospective study," *Fertility and Sterility*, vol. 90, no. 3, pp. 513–520, 2008.

[40] C. L. Haggerty, P. A. Totten, S. G. Astete et al., "Failure of cefoxitin and doxycycline to eradicate endometrial *Mycoplasma genitalium* and the consequence for clinical cure of pelvic inflammatory disease," *Sexually Transmitted Infections*, vol. 84, no. 5, pp. 338–342, 2008.

[41] V. L. Short, P. A. Totten, R. B. Ness, S. G. Astete, S. F. Kelsey, and C. L. Haggerty, "Clinical presentation of *Mycoplasma genitalium* infection versus *Neisseria gonorrhoeae* infection among women with pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 48, no. 1, pp. 41–47, 2009.

[42] R. B. Ness, D. E. Soper, R. L. Holley et al., "Effectiveness of inpatient and outpatient treatment strategies for women with pelvic inflammatory disease: results from the pelvic inflammatory disease evaluation and clinical health (PEACH) randomized trial," *American Journal of Obstetrics and Gynecology*, vol. 186, no. 5, pp. 929–937, 2002.

[43] R. B. Ness, S. L. Hillier, K. E. Kip et al., "Bacterial vaginosis and risk of pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 104, no. 4, pp. 761–769, 2004.

[44] D. E. Soper, N. J. Brockwell, H. P. Dalton, and D. Johnson, "Observations concerning the microbial etiology of acute salpingitis," *American Journal of Obstetrics and Gynecology*, vol. 170, no. 4, pp. 1008–1017, 1994.

[45] S. L. Hillier, N. B. Kiviat, S. E. Hawes et al., "Role of bacterial vaginosis-associated microorganisms in endometritis," *American Journal of Obstetrics and Gynecology*, vol. 175, no. 2, pp. 435–441, 1996.

[46] D. A. Eschenbach, S. Hillier, C. Critchlow et al., "Diagnosis and clinical manifestations of bacterial vaginosis," *American Journal of Obstetrics and Gynecology* , vol. 158, pp. 819–828, 1988.

[47] A. P. Korn, G. Bolan, N. Padian, M. Ohm-Smith, J. Schachter, and D. V. Landers, "Plasma cell endometritis in women with symptomatic bacterial vaginosis," *Obstetrics and Gynecology*, vol. 85, no. 3, pp. 387–390, 1995.

[48] J. F. Peipert, A. B. Montagno, A. S. Cooper, and C. J. Sung, "Bacterial vaginosis as a risk factor for upper genital tract infection," *American Journal of Obstetrics and Gynecology*, vol. 177, no. 5, pp. 1184–1187, 1997.

[49] A. P. Korn, N. A. Hessol, N. S. Padian et al., "Risk factors for plasma cell endometritis among women with cervical *Neisseria gonorrhoeae*, cervical *Chlamydia trachomatis*, or bacterial vaginosis," *American Journal of Obstetrics and Gynecology*, vol. 178, no. 5, pp. 987–990, 1998.

[50] R. L. Sweet, "Role of bacterial vaginosis in pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 20, no. 2, pp. S271–S275, 1995.

[51] R. B. Ness, K. E. Kip, S. L. Hillier et al., "A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease," *American Journal of Epidemiology*, vol. 162, no. 6, pp. 585–590, 2005.

[52] J. K. Hebb, C. R. Cohen, S. G. Astete, E. A. Bukusi, and P. A. Totten, "Detection of novel organisms associated with salpingitis, by use of 16S rDNA polymerase chain reaction," *Journal of Infectious Diseases*, vol. 190, no. 12, pp. 2109–2120, 2004.

[53] L. E. Manhart, C. W. Critchlow, K. K. Holmes et al., "Mucopurulent cervicitis and *Mycoplasma genitalium*," *Journal of Infectious Diseases*, vol. 187, no. 4, pp. 650–657, 2003.

[54] J. Pépin, A.-C. Labbé, N. Khonde et al., "*Mycoplasma qenitalium*: an organism commonly associated with cervicitis among west African sex workers," *Sexually Transmitted Infections*, vol. 81, no. 1, pp. 67–72, 2005.

[55] B. R. Moller, D. Taylor-Robinson, P. M. Furr, and E. A. Freundt, "Acute upper genital-tract disease in female monkeys provoked experimentally by *Mycoplasma genitalium*," *British Journal of Experimental Pathology*, vol. 66, no. 4, pp. 417–426, 1985.

[56] A. Barzynska, P. Funch, J. Fedder, H. J. Knudsen, S. Birkelund, and G. Christiansen, "Morphology of human Fallopian tubes after infection with *Mycoplasma genitalium* and *Mycoplasma hominis*: In vitro organ culture study," *Human Reproduction*, vol. 22, no. 4, pp. 968–979, 2007.

[57] R. L. Sweet and R. S. Gibbs, "Pelvic inflammatory disease," in *Infectious Diseases of the Female Genital Tract*, pp. 220–244, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 2009.

[58] D. L. Patton, Y. T. C. Sweeney, and C. C. Kuo, "Demonstration of delayed hypersensitivity in *Chlamydia trachomatis* salpingitis in monkeys: a pathogenic mechanism of tubal damage," *Journal of Infectious Diseases*, vol. 169, no. 3, pp. 680–683, 1994.

[59] R. C. Brunham and R. W. Peeling, "*Chlamydia trachomatis* antigens: role in immunity and pathogenesis," *Infectious Agents and Disease*, vol. 3, no. 5, pp. 218–233, 1994.

[60] L. O. Eckert, S. E. Hawes, P. Wölner-Hanssen et al., "Prevalence and correlates of antibody to chlamydial heat shock protein in women attending sexually transmitted disease clinics and women with confirmed pelvic inflammatory disease," *Journal of Infectious Diseases*, vol. 175, no. 6, pp. 1453–1458, 1997.

[61] R. L. Sweet, J. Schachter, and M. O. Robbie, "Failure of β-lactam antibiotics to eradicate *Chlamydia trachomatis* in the endometrium despite apparent clinical cure of acute salpingitis," *Journal of the American Medical Association*, vol. 250, no. 19, pp. 2641–2645, 1983.

[62] W. R. Crombleholme, J. Schachter, M. Ohm-Smith, J. Luft, R. Whidden, and R. L. Sweet, "Efficacy of single-agent therapy for the treatment of acute pelvic inflammatory disease with ciprofloxacin," *American Journal of Medicine*, vol. 87, no. 5, pp. 142S–147S, 1989.

[63] C. K. Walker, J. G. Kahn, A. E. Washington, H. B. Peterson, and R. L. Sweet, "Pelvic inflammatory disease: metaanalysis of antimicrobial regimen efficacy," *Journal of Infectious Diseases*, vol. 168, no. 4, pp. 969–978, 1993.

[64] C. K. Walker and R. L. Sweet, "Management," in *Pelvic Inflammatory Disease*, R. L. Sweet and H. C. Wiesenfeld, Eds., pp. 141–156, Taylor & Francis, London, UK, 2006.

[65] R. B. Ness, S. L. Hillier, K. E. Kip et al., "Douching, pelvic inflammatory disease, and incident gonococcal and chlamydial genital infection in a cohort of high-risk women," *American Journal of Epidemiology*, vol. 161, no. 2, pp. 186–195, 2005.

[66] L. O. Eckert, S. S. Thwin, S. L. Millier, N. B. Kiviat, and D. A. Eschenbach, "The antimicrobial treatment of subacute endometritis: a proof of concept study," *American Journal of Obstetrics and Gynecology*, vol. 190, no. 2, pp. 305–313, 2004.

[67] S. M. Lareau and R. H. Beigi, "Pelvic inflammatory disease and tubo-ovarian abscess," *Infectious Disease Clinics of North America*, vol. 22, no. 4, pp. 693–708, 2008.

[68] R. L. Sweet, "Sexually transmitted diseases. Pelvic inflammatory disease and infertility in women," *Infectious Disease Clinics of North America*, vol. 1, no. 1, pp. 199–215, 1987.

[69] D. Eschenbach, "Treatment of pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 44, no. 7, pp. 961–963, 2007.

[70] L. Svensson, P. A. Mardh, and L. Westrom, "Infertility after acute salpingitis with special reference to *Chlamydia trachomatis*," *Fertility and Sterility*, vol. 40, no. 3, pp. 322–329, 1983.

[71] S. D. Hillis, R. Joesoef, P. A. Marchbanks, J. N. Wasserheit, W. Cates, and L. Westrom, "Delayed care of pelvic inflammatory disease as a risk factor for impaired fertility," *American Journal of Obstetrics and Gynecology*, vol. 168, no. 5, pp. 1503–1509, 1993.

[72] K. J. Smith, R. B. Ness, H. C. Wiesenfeld, and M. S. Roberts, "Cost-effectiveness of alternative outpatient pelvic inflammatory disease treatment strategies," *Sexually Transmitted Diseases*, vol. 34, no. 12, pp. 960–966, 2007.

[73] K. E. Aldridge, D. Ashcraft, K. Cambre, C. L. Pierson, S. G. Jenkins, and J. E. Rosenblatt, "Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella, Fusobacterium, Porphyromonas,* and *Peptostreptococcus* species," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 4, pp. 1238–1243, 2001.

[74] R. H. Beigi, M. N. Austin, L. A. Meyn, M. A. Krohn, and S. L. Hillier, "Antimicrobial resistance associated with the treatment of bacterial vaginosis," *American Journal of Obstetrics and Gynecology*, vol. 191, no. 4, pp. 1124–1129, 2004.

[75] G. W. Amsden, "Anti-inflammatory effects of macrolides—An underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions?" *Journal of Antimicrobial Chemotherapy*, vol. 55, no. 1, pp. 10–21, 2005.

[76] D. L. Patton, Y. T. Cosgrove Sweeney, and W. E. Stamm, "Significant reduction in inflammatory response in the macaque model of chlamydial pelvic inflammatory disease with azithromycin treatment," *Journal of Infectious Diseases*, vol. 192, no. 1, pp. 129–135, 2005.

[77] C. D. Bevan, G. L. Ridgway, and C. D. Rothermel, "Efficacy and safety of azithromycin as monotherapy or combined with metronidazole compared with two standard multidrug regimens for the treatment of acute pelvic inflammatory disease," *Journal of International Medical Research*, vol. 31, no. 1, pp. 45–54, 2003.

[78] R. B. Ness, G. Trautmann, H. E. Richter et al., "Effectiveness of treatment strategies of some women with pelvic inflammatory disease: a randomized trial," *Obstetrics and Gynecology*, vol. 106, no. 3, pp. 573–580, 2005.

[79] J. A. McGregor, W. R. Crombleholme, E. Newton, R. L. Sweet, R. Tuomala, and R. S. Gibbs, "Randomized comparison of ampicillin-sulbactam to cefoxitin and doxycycline or clindamycin and gentamicin in the treatment of pelvic inflammatory disease or endometritis," *Obstetrics and Gynecology*, vol. 83, no. 6, pp. 998–1004, 1994.

[80] R. F. Savaris, L. M. Teixeira, T. G. Torres, M. I. A. Edelweiss, J. Moncada, and J. Schachter, "Comparing ceftriaxone plus azithromycin or doxycycline for pelvic inflammatory disease: a randomized controlled trial," *Obstetrics and Gynecology*, vol. 110, no. 1, pp. 53–60, 2007.

[81] L. Yip, P. J. Sweeny, and B. F. Bock, "Acute suppurative salpingitis with concomitant intrauterine pregnancy," *American Journal of Emergency Medicine*, vol. 11, no. 5, pp. 476–479, 1993.

[82] A. C. Blanchard, J. G. Pastorek, and T. Weeks, "Pelvic inflammatory disease during pregnancy," *Southern Medical Journal*, vol. 80, no. 11, pp. 1363–1365, 1987.

[83] D. L. Hemsel, W. J. Ledger, M. Martens, G. R. G. Monif, N. G. Osborne, and J. L. Thomason, "Concerns regarding the centers for disease control's published guidelines for pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 32, no. 1, pp. 103–107, 2001.

[84] A. P. Korn, D. V. Landers, J. R. Green, and R. L. Sweet, "Pelvic inflammatory disease in human immunodeficiency virus-infected women," *Obstetrics and Gynecology*, vol. 82, no. 5, pp. 765–768, 1993.

[85] K. L. Irwin, A. C. Moorman, M. J. O'Sullivan et al., "Influence of human immunodeficiency virus infection on pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 95, no. 4, pp. 525–534, 2000.

[86] C. R. Cohen, "Effect of human immunodeficiency virus type 1 infection upon acute salpingitis: a laparoscopic study," *Journal of Infectious Diseases*, vol. 178, no. 5, pp. 1352–1358, 1998.

[87] E. A. Bukusi, C. R. Cohen, C. E. Stevens et al., "Effects of human immunodeficiency virus 1 infection on microbial origins of pelvic inflammatory disease and on efficacy of ambulatory oral therapy," *American Journal of Obstetrics and Gynecology*, vol. 181, no. 6, pp. 1374–1381, 1999.

[88] N. R. Mugo, J. A. Kiehlbauch, R. Nguti et al., "Effect of human immunodeficiency virus-1 infection on treatment outcome of acute salpingitis," *Obstetrics and Gynecology*, vol. 107, no. 4, pp. 807–812, 2006.

[89] G. Soderberg and S. Lindgren, "Influence of an intrauterine device on the course of an acute salpingitis," *Contraception*, vol. 24, no. 2, pp. 137–143, 1981.

[90] S. Altunyurt, N. Demir, and C. Posaci, "A randomized controlled trial of coil removal prior to treatment of pelvic inflammatory disease," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 107, no. 1, pp. 81–84, 2003.

[91] R. Hamasuna, Y. Osada, and J. S. Jensen, "Antibiotic susceptibility testing of *Mycoplasma genitalium* by TaqMan 5′ nuclease real-time PCR," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 12, pp. 4993–4998, 2005.

[92] P. Horner, B. Thomas, C. B. Gilroy, M. Egger, and D. Taylor-Robinson, "Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in acute and chronic nongonococcal urethritis," *Clinical Infectious Diseases*, vol. 32, no. 7, pp. 995–1003, 2001.

[93] E. Jernberg, A. Moghaddam, and H. Moi, "Azithromycin and moxifloxacin for microbiological cure of *Mycoplasma genitalium* infection: an open study," *International Journal of STD and AIDS*, vol. 19, no. 10, pp. 676–679, 2008.

[94] C. S. Bradshaw, M. Y. Chen, and C. K. Fairley, "Persistence of *Mycoplasma genitalium* following azithromycin therapy," *PLoS ONE*, vol. 3, no. 11, Article ID e3618, 2008.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 754060, 5 pages
doi:10.1155/2011/754060

Review Article

Chlamydia trachomatis Infection Control Programs: Lessons Learned and Implications for Vaccine Development

Jean M. Chavez, Rodolfo D. Vicetti Miguel, and Thomas L. Cherpes

Department of Pediatrics, University of Pittsburgh School of Medicine, Rangos Research Center, Room 9123, 4401 Penn Avenue, Pittsburgh, PA 15224, USA

Correspondence should be addressed to Thomas L. Cherpes, cherpestl@upmc.edu

Received 15 July 2011; Accepted 29 August 2011

Academic Editor: Richard L. Sweet

Chlamydia trachomatis control efforts that enhance detection and treatment of infected women may paradoxically increase susceptibility of the population to infection. Conversely, these surveillance programs lower incidences of adverse sequelae elicited by genital tract infection (e.g., pelvic inflammatory disease and ectopic pregnancy), suggesting enhanced identification and eradication of *C. trachomatis* simultaneously reduces pathogen-induced upper genital tract damage and abrogates formation of protective immune responses. In this paper, we detail findings from *C. trachomatis* infection control programs that increase our understanding of chlamydial immunoepidemiology and discuss their implications for prophylactic vaccine design.

1. Introduction

An estimated 90 million individuals sexually acquire *Chlamydia trachomatis* infection each year (including 45 million in Asia, 15 million in Africa, and 4 million in the United States [1]), and in all these geographic regions the highest prevalences of chlamydial genital tract infection are found among adolescents and young adults [2]. Although *C. trachomatis* is a common cause of male nongonococcal urethritis [3], female genital tract infections represent more significant threats to reproductive health. Morbidities associated with *C. trachomatis* genital tract infections in women include pelvic inflammatory disease (PID) and its sequelae of chronic pelvic pain, ectopic pregnancy, and tubal infertility [4].

2. Shortcomings and Benefits of Infection Control Programs

During the past 25 years, many areas in Europe and North America implemented infection control programs to reduce sexual transmission of *C. trachomatis* [5]. These programs typically relied upon widespread screening and prompt treatment of asymptomatic individuals as a conduit for decreased population infectivity [6–8]. Applying these principles, one regional United States program reduced *C. trachomatis* prevalence 60% among young women during the first 9 years of its existence [9]. However, similar screening and treatment of young women in this region during the succeeding 7 years was associated with a 46% increase in chlamydial positivity [10]. This scenario was repeated in British Columbia—after introduction of a *C. trachomatis* infection control program case rates fell from 216 to 104 cases per 100 000 individuals but then steadily climbed to 193 cases per 100 000 individuals [11]. In fact, nearly all countries implementing large-scale chlamydial control programs have reported increased case report numbers despite ongoing control efforts [12–14], suggesting expanded earlier treatment may enhance population susceptibility to *C. trachomatis* infection. Although the higher prevalences of *C. trachomatis* seen in areas with active surveillance may have been sequelae to increased screening of higher risk women or increased use of more sensitive diagnostic tests, at least in 1 such surveillance program higher prevalence appeared to reflect actual increases in chlamydial positivity [15]. Providing stronger support for the supposition that *C. trachomatis* infection control programs increase population susceptibility to infection, British Columbia saw

reinfection rates rise from 9.7 to 53.2 cases per 100,000 individuals in the midst of widespread control efforts [11, 15].

Concurrent with higher incidences of reinfection, however, *C. trachomatis* control programs have reduced genital tract complications elicited by infection. For example, concomitant with steady increases in *C. trachomatis* case numbers, health officials in San Francisco County, Calif, observed dramatic decreases in PID and ectopic pregnancy cases [16]. Earlier diagnosis and treatment of genital tract chlamydial infections was also associated with sharp reductions in ectopic pregnancy rates in Norway and Sweden [17, 18]. Despite the substantially increased rates of *C. trachomatis* infection documented in British Columbia [11], surveillance data demonstrated robust decreases in annual case numbers and rates of PID, ectopic pregnancy, and tubal factor infertility [19]. Taken together, these data imply that enhanced detection and earlier treatment of infected women achieved upon implementation of *C. trachomatis* infection control programs may have been responsible for reduced incidences of the adverse outcomes associated with ascension of this pathogen into the upper genital tract. These data further imply that persistent *C. trachomatis* infection, not simply acquisition or reinfection, may be the scenario most likely responsible for development of PID, ectopic pregnancy, and tubal factor infertility.

3. *C. trachomatis* Control Programs and the Arrested Immunity Hypothesis

Seminal investigations performed in British Columbia allowed Brunham et al. to first posit that *C. trachomatis* infection control programs increase population susceptibility to reinfection [11]. Their "altered immunity" hypothesis states that development of protective immune responses against *C. trachomatis* is abrogated by earlier detection and treatment of infected individuals and further argues this interrupted development of protective immunity increases the likelihood of reinfection. Evidence supporting this proposed linkage between expanded earlier treatment and increased population susceptibility to infection has been provided by both experimental and clinical investigations. Compared to untreated mice, humoral immune responses were impaired in the vaginas of mice administered doxycycline within the first 10 days of primary intravaginal chlamydial infection. Moreover, these same antibiotic-treated mice were also less protected from chlamydial reinfection [20]. Taken together, these data suggest that accelerated eradication of chlamydia from the genital tract that was mediated by doxycycline therapy may have hampered the development of protective immune responses.

The durability of *C. trachomatis* infection among many women not receiving antichlamydial antibiotics implies protracted courses of infection may be needed for development of sterilizing immunity, while providing further support for the validity of the altered immunity hypothesis. For example, an annual clearance rate of 45% among asymptomatic Dutch women not receiving antimicrobial therapy suggested sterilizing immunity is often not achieved during the first year of a chlamydial genital tract infection [21]. A similar rate of clearance was seen in Colombia where 54% (44/82) of women not receiving antichlamydial antibiotics cleared asymptomatic genital tract infection during the first year after initial diagnosis; however *C. trachomatis* infection persisted in only 6% of this cohort after 4 years of followup [22]. These results indicate the development of sterilizing immunity against genital tract chlamydial infection is most often measured in months or years, while long-term presence of the organism in the absence of overt inflammatory symptoms highlights the highly successful parasitic relationship *C. trachomatis* has achieved with its human hosts. In addition to these natural history studies, clinical data in support of the altered immune hypothesis was generated upon completion of a C. *trachomatis* seroprevalence study enrolling 8 000 pregnant Finnish women. During the same period of time in this country in which dramatic increases in the frequency of genital tract chlamydial infection were observed [23], this study reported a 51% decrease in the prevalence of positive serum IgG antibody titers against *C. trachomatis* major outer membrane protein (MOMP) among women less than 23 years of age and a 65% decrease in positive titers among 23–28-year-old women [24]. These findings may indicate that in some women chlamydial MOMP-specific humoral responses are transitory or, conversely, that humoral responses against *C. trachomatis* are slow to develop and that earlier identification and treatment of infection impeded the development of humoral immunity. Although more speculative, it is also possible that decreasing seroprevalence of *C. trachomatis* in Finland contributed to increased population susceptibility to reinfection. This latter clinical scenario is consistent with experimental data demonstrating that chlamydial-specific antibodies were integral for protection of female mice from genital tract reinfection [25]. Further clinical investigation, however, will be needed to determine the strength of the associations between enhanced detection and treatment, impaired humoral immune responses, and increases in susceptibility to *C. trachomatis* genital tract reinfection.

4. Implications for *C. trachomatis* Vaccine Development

Widespread *C. trachomatis* infection control programs reduce incidences of PID and its adverse sequelae [19, 26], but are associated with increased population susceptibility to infection. These seemingly contradictory observations interestingly help illuminate the immunoepidemiology of *C. trachomatis* infection. Although some conclusions that we draw from these epidemiological investigations remain conjectural, increased population susceptibility to chlamydial infection seen concomitantly with decreases in genital tract complications of infection indicates the following: (1) this obligate intracellular pathogen is weakly antigenic; (2) the organism requires persistent infection to elicit upper genital tract damage; (3) primary infection is associated with host immune responses that are suboptimal for immediate pathogen clearance but unlikely to damage vital upper genital tract architecture and/or this organism employs

immunoevasion strategies that promote establishment of asymptomatic but persistent infection.

Consistent with the notion of low antigenicity, *C. trachomatis* infections of the female genital tract often remain asymptomatic. Cell walls of *C. trachomatis*, like other Gram-negative bacteria, contain lipopolysaccharide (LPS), a molecule that stimulates multiple responses in infected tissue including increased secretion of pro-inflammatory cytokines, macrophage activation, and increased expression of endothelial leukocyte adhesion molecules. Ex vivo assays show that LPS is primarily responsible for the increased production of tumor necrosis factor (TNF) elicited by *C. trachomatis* elementary bodies, even though chlamydia LPS is 100-fold less potent for the production of this key pro-inflammatory cytokine than the LPS isolated from *Neisseria* species [27]. Therefore, epidemiological data suggesting that the presence of persistent genital tract infection promotes chlamydial disease expression is consistent with this ability of *C. trachomatis* to elicit less robust inflammatory responses [28]. In other words, pelvic inflammatory disease, fallopian tube scarring, and tubal infertility may more frequently result from the presence of chronic, albeit mild inflammation. Although persistent infection may be responsible for increased chlamydial disease expression, the specific immune responses that evoke this upper genital tract damage remain unknown. Mouse and nonhuman primate studies suggest IFN-γ-producing $CD4^+$ T cells are needed for clearance of chlamydial infections, and the generation of appropriate type 1 immunity is often considered to be an integral component of any vaccine conferring protection against chlamydial disease expression [29]. On the other hand, increased IFN-γ production, in conjunction with other type 1 responses, may promote immunopathological responses and increase the likelihood of fallopian tube scarring and tubal factor infertility. Since genital tract *C. trachomatis* infections in nature are weakly antigenic, it is at least a theoretical concern that chlamydial vaccines that confer protection via generation of robust, more durable memory T cell responses could also elicit immunopathological damage if repetitive bouts of inflammation were elicited upon subsequent exposures to the organism.

C. trachomatis is known to have evolved exclusively as a human pathogen and fact which may explain why infection usually occurs in the absence of overt inflammation. Strategies developed by this intracellular pathogen to avoid host detection or clearance include replication within membrane-bound inclusions [30], suppression of class I and II major histocompatibility complex molecule expression by infected cells [31], and its ability to capture indole to escape IFN-γ-mediated tryptophan starvation [32]. A high-frequency asymptomatic *C. trachomatis* infection, on the other hand, may be the consequence of host responses that evolved to minimize collateral damage to delicate upper genital tract structures. In support of this hypothesis, we recently saw that *C. trachomatis* genital tract infection was associated with increased numbers of endometrial $CD4^+$ T cells, B cells, and plasma cells [33], suggesting there is a predilection for *C. trachomatis* infection to polarize endometrial inflammation toward type 2 immunity. Although type 1 immune responses are capable of clearing *C. trachomatis*, type 2 responses may have been selected as a safer alternative to more damaging type 1 responses in the upper genital tract [34]. Whether type 2 endometrial inflammation is associated with enhanced or impaired chlamydial clearance or higher or lower chances of immunopathological tissue damage remains unknown, but resolution of these uncertainties is essential for proper vaccine development. The reported linkage between enhanced early detection, decreased chlamydia-specific antibody prevalence, and increased chlamydial reinfection rates does not establish causality between impaired humoral immune responses and increased susceptibility to infection, but does provide the impetus for more complete understanding of the immune responses that may impact chlamydial vaccine efficacy.

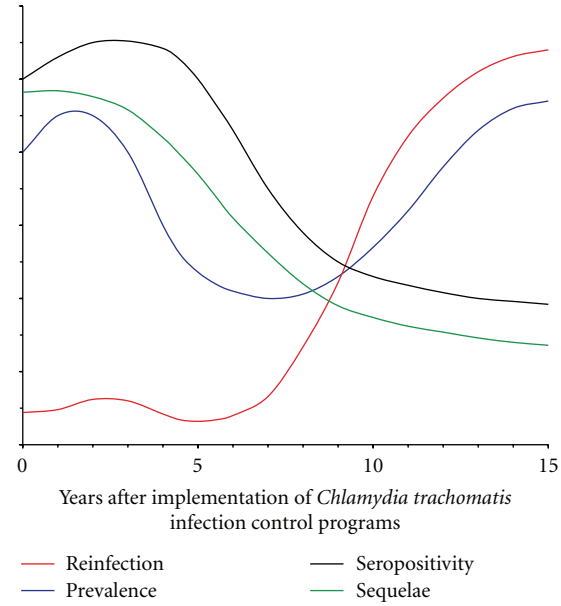


Figure 1: Schema summarizing outcomes associated with *Chlamydia trachomatis* genital tract infection control programs. These outcomes include increased number of chlamydial cases, increased rates of chlamydial reinfection, decreased detection of chlamydial-specific serum antibodies, and decreased rates of pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility. Although no causality between these observed outcomes has been established, chlamydial vaccine development will require better delineation of the linkage between enhanced early treatment and diminished antichlamydial humoral immunity, increased susceptibility to infection, and lower incidences of adverse reproductive tract sequelae.

In conclusion, this brief paper summarized the findings from *C. trachomatis* infection control programs that alter our understanding of the immunoepidemiology of chlamydial genital tract infection (Figure 1). Although observations from these programs suggest increased duration of infection is a risk factor for the development of PID, understanding of the specific host genetic variations and immune responses that promote genital tract damage awaits further investigation. Additional work is also needed to better inform chlamydial vaccine development, as more comprehensive

understanding of the immune responses that protect against *C. trachomatis* acquisition and reinfection and prevent or elicit PID development must be achieved before clinical vaccination trials can be safely initiated.

Acknowledgments

Authors acknowledge Dr. John F. Alcorn for critical paper review. Paper preparation was supported by funds provided by the National Institute of Allergy and Infectious Diseases (Grant U19AI084024).

References

[1] R. C. Brunham and J. Rey-Ladino, "Immunology of chlamydia infection: implications for a *Chlamydia trachomatis* vaccine," *Nature Reviews Immunology*, vol. 5, no. 2, pp. 149–161, 2005.

[2] Centers for Disease Control and Prevention (CDC), "CDC grand rounds: Chlamydia prevention: challenges and strategies for reducing disease burden and sequelae," *Morbidity and Mortality Weekly Report*, vol. 60, no. 12, pp. 370–373, 2011.

[3] J. R. Schwebke, A. Rompalo, S. Taylor et al., "Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens—a randomized clinical trial," *Clinical Infectious Diseases*, vol. 52, no. 2, pp. 163–170, 2011.

[4] J. Paavonen and W. Eggert-Kruse, "*Chlamydia trachomatis*: impact on human reproduction," *Human Reproduction Update*, vol. 5, no. 5, pp. 433–447, 1999.

[5] S. L. Gottlieb, R. C. Brunham, G. I. Byrne, D. H. Martin, F. Xu, and S. M. Berman, "Introduction: the natural history and immunobiology of *Chlamydia trachomatis* genital infection and implications for Chlamydia control," *Journal of Infectious Diseases*, vol. 201, supplement 2, pp. S85–S87, 2010.

[6] S. D. Hillis, A. Nakashima, L. Amsterdam et al., "The impact of a comprehensive chlamydia prevention program in Wisconsin," *Family Planning Perspectives*, vol. 27, no. 3, pp. 108–111, 1995.

[7] C. Panchaud, S. Singh, D. Feivelson, and J. E. Darroch, "Sexually transmitted diseases among adolescents in developed countries," *Family Planning Perspectives*, vol. 32, no. 1, pp. 24–45, 2000.

[8] F. Kamwendo, L. Forslin, L. Bodin, and D. Danielsson, "Decreasing incidences of gonorrhea- and Chlamydia-associated acute pelvic inflammatory disease: a 25-year study from an urban area of Central Sweden," *Sexually Transmitted Diseases*, vol. 23, no. 5, pp. 384–391, 1996.

[9] Centers for Disease Control and Prevention (CDC), "*Chlamydia trachomatis* genital infections—United States," *Morbidity and Mortality Weekly Report*, vol. 46, pp. 193–198, 1997.

[10] D. Fine, L. Dicker, D. Mosure, and S. Berman, "Increasing chlamydia positivity in women screened in family planning clinics: do we know why?" *Sexually Transmitted Diseases*, vol. 35, no. 1, pp. 47–52, 2008.

[11] R. C. Brunham, B. Pourbohloul, S. Mak, R. White, and M. L. Rekart, "The unexpected impact of a *Chlamydia trachomatis* infection control program on susceptibility to reinfection," *Journal of Infectious Diseases*, vol. 192, no. 10, pp. 1836–1844, 2005.

[12] Project SCREen, Review of chlamydia control activities in EU countries, Final report, 2011, http://www.ecdc.europa.eu/en/publications/Publications/0805_TER_Review_of_Chlamydia_Control_Activities.pdf.

[13] Centers for Disease Control and Prevention, "Sexually transmitted disease surveillance 2007 supplement, chlamydia prevalence monitoring project annual report 2007," Tech. Rep., Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, Ga, USA, January 2009.

[14] M. L. Rekart and R. C. Brunham, "Epidemiology of chlamydial infection: are we losing ground?" *Sexually Transmitted Infections*, vol. 84, no. 2, pp. 87–91, 2008.

[15] R. C. Brunham, B. Pourbohloul, S. Mak, R. White, and M. L. Rekart, "Reply to Hagdu and to Moss et al," *Journal of Infectious Diseases*, vol. 193, no. 9, pp. 1338–1339, 2006.

[16] N. J. Moss, K. Ahrens, C. K. Kent, and J. D. Klausner, "The decline in clinical sequelae of genital *Chlamydia trachomatis* infection supports current control strategies," *Journal of Infectious Diseases*, vol. 193, no. 9, pp. 1336–1338, 2006.

[17] I. J. Bakken and F. E. Skjeldestad, "Time trends in ectopic pregnancies in a Norwegian county 1970–2004—a population-based study," *Human Reproduction*, vol. 21, no. 12, pp. 3132–3136, 2006.

[18] F. Kamwendo, L. Forslin, L. Bodin, and D. Danielsson, "Epidemiology of ectopic pregnancy during a 28 year period and the role of pelvic inflammatory disease," *Sexually Transmitted Infections*, vol. 76, no. 1, pp. 28–32, 2000.

[19] R. C. Brunham and M. L. Rekart, "Considerations on *Chlamydia trachomatis* disease expression," *FEMS Immunology and Medical Microbiology*, vol. 55, no. 2, pp. 162–166, 2009.

[20] H. Su, R. Morrison, R. Messer, W. Whitmire, S. Hughes, and H. D. Caldwell, "The effect of doxycycline treatment on the development of protective immunity in a murine model of chlamydial genital infection," *Journal of Infectious Diseases*, vol. 180, no. 4, pp. 1252–1258, 1999.

[21] S. A. Morré, A. J. van den Brule, L. Rozendaal et al., "The natural course of asymptomatic *Chlamydia trachomatis* infections: 45% Clearance and no development of clinical PID after one-year follow-up," *International Journal of STD and AIDS*, vol. 13, supplement 2, pp. 12–18, 2002.

[22] M. Molano, C. J. L. M. Meijer, E. Weiderpass et al., "The natural course of *Chlamydia trachomatis* infection in asymptomatic Colombian women: a 5-year follow-up study," *Journal of Infectious Diseases*, vol. 191, no. 6, pp. 907–916, 2005.

[23] E. Lyytikäinen, M. Kaasila, E. Hiltunen-Back et al., "A discrepancy of *Chlamydia trachomatis* incidence and prevalence trends in Finland 1983–2003," *BMC Infectious Diseases*, vol. 8, article 169, 2008.

[24] E. Lyytikäinen, M. Kaasila, P. Koskela et al., "*Chlamydia trachomatis* seroprevalence atlas of Finland 1983–2003," *Sexually Transmitted Infections*, vol. 84, no. 1, pp. 19–22, 2008.

[25] S. G. Morrison and R. P. Morrison, "A predominant role for antibody in acquired immunity to chlamydial genital tract reinfection," *Journal of Immunology*, vol. 175, no. 11, pp. 7536–7542, 2005.

[26] F. Kamwendo, L. Forslin, L. Bodin, and D. Danielsson, "Decreasing incidences of gonorrhea- and Chlamydia-associated acute pelvic inflammatory disease: a 25-year study from an urban area of Central Sweden," *Sexually Transmitted Diseases*, vol. 23, no. 5, pp. 384–391, 1996.

[27] R. R. Ingalls, P. A. Rice, N. Qureshi, K. Takayama, G. S. Lin, and D. T. Golenbock, "The inflammatory cytokine response to *Chlamydia trachomatis* infection is endotoxin mediated," *Infection and Immunity*, vol. 63, no. 8, pp. 3125–3130, 1995.

[28] R. C. Brunham and M. L. Rekart, "The arrested immunity hypothesis and the epidemiology of chlamydia control," *Sexually Transmitted Diseases*, vol. 35, no. 1, pp. 53–54, 2008.

[29] I. Miyairi, K. H. Ramsey, and D. L. Patton, "Duration of untreated Chlamydial genital infection and factors associated with clearance: review of animal studies," *Journal of Infectious Diseases*, vol. 201, supplement 2, pp. S96–S103, 2010.

[30] K. A. Fields and T. Hackstadt, "The chlamydial inclusion: escape from the endocytic pathway," *Annual Review of Cell and Developmental Biology*, vol. 18, pp. 221–245, 2002.

[31] G. Zhong, L. Liu, T. Fan, P. Fan, and H. Ji, "Degradation of transcription factor RFX5 during the inhibition of both constitutive and interferon γ-inducible major histocompatibility complex class I expression in chlamydia-infected cells," *Journal of Experimental Medicine*, vol. 191, no. 9, pp. 1525–1534, 2000.

[32] H. D. Caldwell, H. Wood, D. Crane et al., "Polymorphisms in *Chlamydia trachomatis* tryptophan synthase genes differentiate between genital and ocular isolates," *Journal of Clinical Investigation*, vol. 111, no. 11, pp. 1757–1769, 2003.

[33] S. D. Reighard, R. L. Sweet, C. V. Miguel et al., "Endometrial leukocyte subpopulations associated with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* genital tract infection," *American Journal of Obstetrics and Gynecology*, vol. 205, no. 4, pp. 324.e1–324.e7, 2011.

[34] A. L. Graham, J. E. Allen, and A. F. Read, "Evolutionary causes and consequences of immunopathology," *Annual Review of Ecology, Evolution, and Systematics*, vol. 36, pp. 373–397, 2005.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 428351, 7 pages
doi:10.1155/2011/428351

Research Article

Detection of Pelvic Inflammatory Disease: Development of an Automated Case-Finding Algorithm Using Administrative Data

Catherine L. Satterwhite,[1] Onchee Yu,[2] Marsha A. Raebel,[3] Stuart Berman,[1] Penelope P. Howards,[4] Hillard Weinstock,[1] David Kleinbaum,[4] and Delia Scholes[2]

[1] *Division of STD Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop E-02, Atlanta, GA 30333, USA*
[2] *Group Health Research Institute, Group Health Cooperative, Seattle, WA 98101, USA*
[3] *Institute for Health Research, Kaiser Permanente, Denver, CO 80231, USA*
[4] *Department of Epidemiology, Emory University, Atlanta, GA 30322, USA*

Correspondence should be addressed to Catherine L. Satterwhite, col8@cdc.gov

Received 28 August 2011; Accepted 27 September 2011

Academic Editor: Thomas Cherpes

ICD-9 codes are conventionally used to identify pelvic inflammatory disease (PID) from administrative data for surveillance purposes. This approach may include non-PID cases. To refine PID case identification among women with ICD-9 codes suggestive of PID, a case-finding algorithm was developed using additional variables. Potential PID cases were identified among women aged 15–44 years at Group Health (GH) and Kaiser Permanente Colorado (KPCO) and verified by medical record review. A classification and regression tree analysis was used to develop the algorithm at GH; validation occurred at KPCO. The positive predictive value (PPV) for using ICD-9 codes alone to identify clinical PID cases was 79%. The algorithm identified PID appropriate treatment and age 15–25 years as predictors. Algorithm sensitivity (GH = 96.4%; KPCO = 90.3%) and PPV (GH = 86.9%; KPCO = 84.5%) were high, but specificity was poor (GH = 45.9%; KPCO = 37.0%). In GH, the algorithm offered a practical alternative to medical record review to further improve PID case identification.

1. Introduction

An estimated 770,000 cases of pelvic inflammatory disease (PID) are diagnosed annually in the United States [1]. PID comprises infection and inflammation of the uterus, fallopian tubes, ovaries, and other adjacent tissue and has multiple infectious etiologies, many of which have been demonstrated to be sexually transmitted, including *Chlamydia trachomatis* [2]. *C. trachomatis* has been isolated in approximately one-quarter of patients with a symptomatic PID diagnosis [3].

PID of any etiology may lead to further adverse outcomes, including tubal-factor infertility, ectopic pregnancy, and chronic pelvic pain [2]; about 10%–20% of PID cases are associated with infertility and ectopic pregnancy [3]. The specific contribution of chlamydia and other infections associated with PID (e.g., *Neisseria gonorrhoeae* and *Mycoplasma genitalium*) to each of these adverse outcomes is unknown [4, 5]. However, among infertile couples using assisted reproductive therapy, 10–20% are diagnosed with tubal infertility [6, 7]. In an effort to prevent PID and subsequent infertility, chlamydia screening is recommended for all sexually active women aged <25 years [8, 9]. Prior studies have suggested that screening can reduce the risk of PID development by up to 50% [10, 11].

While monitoring trends in PID is a critical component to quantifying the public health burden of PID and evaluating the impact of chlamydia prevention efforts, PID surveillance is challenging. In the absence of a laboratory-based case definition, PID is diagnosed on the basis of clinical signs and symptoms [12]. The Centers for Disease Control and Prevention (CDC) recommends empiric treatment for PID when young women have lower abdominal pain with no other clear cause, accompanied by either

uterine or adnexal or cervical motion tenderness [8]. Thus, the clinical diagnosis lacks specificity. The "gold standard" for diagnosing tubal infection is laparoscopy, an invasive procedure that is rarely performed in clinical practice [13]. To identify PID cases for research and surveillance purposes, medical record review provides the best method of verifying a clinical diagnosis of PID. In lieu of medical record reviews, ICD-9 codes have been used to identify PID cases from administrative data. A clinical diagnosis of PID may be represented by several ICD-9 codes. The most commonly referenced ICD-9 code, 614.9 (female pelvic inflammatory disease not otherwise specified) has a positive predictive value (PPV) of only 18.1% for the PID surveillance case definition, a substantially stricter definition than the clinical definition used for empiric treatment [14]. When coupled with a positive chlamydia test, the PPV increases to 56%; however, laboratory test results are frequently unavailable in the administrative datasets used to examine PID rates and trends. Information is lacking on the PPV of the multiple ICD-9 codes currently in use for surveillance of acute PID.

Potential PID cases identified from administrative data using ICD-9 codes include some women without PID. To further refine identification of clinically diagnosed PID in the subset of women with an ICD-9 code suggestive of PID, medical record review is preferable but is costly. Applying a PID case-finding algorithm based on additional administrative data to potential PID cases identified from ICD-9 codes may be more practical and allow for more accurate burden and trend ascertainment for PID surveillance activities. The purpose of this analysis was twofold: (1) to determine the PPV of using ICD-9 codes alone to identify PID and (2) to develop a PID case-finding algorithm using administrative data elements to easily identify PID cases among women with PID-related ICD-9 diagnostic codes.

Table 1: ICD-9 codes commonly utilized to identify possible acute pelvic inflammatory disease (PID) and code distribution among potential PID cases sampled from Group Health Cooperative.

ICD-9 Code and Description	Number of potential cases with code* (%)
098.10-Acute GC upper GU tract, site unspecified 098.16-Acute GC endometritis 098.17-Acute GC salpingitis 098.19-Acute GC upper GU tract, other site 098.86-Acute GC peritonitis	5 (1.3)
099.56-Acute CT peritonitis	0 (0.0)
614.0-Acute salpingo-oophoritis 614.5-Acute or unspecified pelvic peritonitis 614.8-Other specified inflammatory disease, female pelvic organs	8 (2.0)
614.2-Salpingitis/oophoritis, not acute or chronic	22 (5.6)
614.3-Acute parametritis/PID	53 (13.5)
614.9-Unspecified inflammatory disease, female pelvic organs	252 (64.1)
615.0-Inflammatory disease of uterus, except cervix	15 (3.8)
615.9-Unspecified inflammatory disease of uterus	80 (20.4)

GC: gonorrhea, GU: genitourinary, CT: chlamydia.
*A single potential PID case may include multiple ICD-9 codes. 393 total potential PID cases were identified, and a total of 435 ICD-9 codes were used.

2. Materials and Methods

Data from two mixed model healthcare organizations, Group Health Cooperative (GH, Seattle, Wash, USA) and Kaiser Permanente Colorado (KPCO, Denver, CO), were used in this analysis. In 2006, approximately 125,000 women between the ages of 15 and 44 years were enrolled in GH, and about 116,000 women of the same ages were enrolled in KPCO. Both organizations maintain extensive automated administrative and clinical data including enrollment information, demographics, health care utilization, diagnoses, procedures, laboratory tests, and pharmacy records on each enrollee.

2.1. Data Collection. A set of 14 ICD-9 codes used in other epidemiologic evaluations of PID was used to identify women with potential PID cases in the GH administrative database (Table 1) [1, 15]. Only codes for PID not identified as chronic were considered, since these cases may be more likely to represent PID cases associated with infectious causes such as chlamydia that could be prevented by screening efforts. PID diagnoses that occurred within 60 days of each other were considered the same PID episode. Using GH data from 2003 to 2007, there were 2,764 total potential PID cases among women aged 15 to 44 years. During this time period, PID rates declined slightly from 568 cases/100,000 person-years to 473/100,000; these data have been described in another publication [16]. From the 2,764 total potential cases, a sample of 393 potential cases was randomly selected for medical record review to determine if the clinical diagnosis was PID. If a woman had multiple PID diagnoses from 2003 to 2007, only the first PID episode was included in the sample. The distribution of ICD-9 codes associated with the 393 potential cases is shown in Table 1; multiple ICD-9 codes could have been selected for the visit associated with each potential PID case.

Determination of the actual PID case status (i.e., clinical diagnosis) was made by reviewing electronic medical records using a structured chart review instrument. Potential PID cases were confirmed as being clinician-diagnosed cases or not based on explicit clinician documentation of PID used in the context of diagnosis during the visit (e.g., "PID," "pelvic inflammatory disease," "pelvic infection," "salpingitis," etc.); PID documentation used for patient evaluation (e.g., "rule-out PID") was not considered. The determination of clinical PID status was made regardless of the clinical signs or symptoms indicated to support such a diagnosis. Cases where the clinical status was uncertain were further reviewed by a study team member (DS).

Table 2: Results from medical record reviews to assess PID cases status at Group Health Cooperative (GH) and Kaiser Permanente Colorado (KPCO).

	PID diagnosis based on medical record review (%)				Total
	PID	Not PID	Uncertain	No information	
GH development dataset	275 (70.0)	74 (18.8)	6 (1.5)	38 (9.7)	393
KPCO validation dataset	349 (69.8)	92 (18.4)	5 (1.0)	54 (10.8)	500

PID: pelvic inflammatory disease.

In addition to ICD-9 diagnosis codes for PID shown in Table 1, other variables potentially associated with PID were extracted from the GH administrative data to be evaluated as potential predictors in the development of the PID case-finding algorithm. These included age at diagnosis, treatment for PID, inpatient admission, whether chlamydia testing was conducted, and other diagnoses occurring 7 days prior to the first PID diagnosis through 7 days after the last PID diagnosis in an episode. These other possible diagnoses were defined by ICD-9 codes and included appendicitis, ovarian cysts, ectopic pregnancy, pyelonephritis, pancreatitis, leiomyoma, and endometriosis. Treatment appropriate for PID was defined as levofloxacin (500 mg orally once per day for 14 days) or ofloxacin (400 mg orally twice per day for 14 days) based on 2006 recommended PID treatment [12]; other possible antimicrobial regimens for PID treatment were also included.

Administrative and medical record data from KPCO were used as an external validation dataset to evaluate the performance of the PID case-finding algorithm in another setting. In the KPCO administrative data from 2003 to 2008, 2,685 potential PID cases among women aged 15 to 44 years were identified using the same ICD-9 codes (Table 1). Of these, 500 were randomly selected for medical record review to determine the clinical PID case status. The same structured chart review instrument that was used in the GH development dataset was used for the medical record review at KPCO. All study procedures received human subjects review and approval at each institution (GH and KPCO).

2.2. Statistical Analysis. A classification and regression tree (CART) analysis was performed to develop a PID case-finding algorithm using the GH dataset. CART has previously been used to improve ectopic pregnancy case finding and to identify diabetes cases [17, 18]. The algorithm goal in this analysis was to identify additional widely-available variables from administrative data that would aid in predicting clinical PID cases as defined by the medical record review. CART is a nonparametric, binary recursive partitioning method that builds a decision tree or a classification algorithm by splitting data into two groups at each branch (or "node") [19]. Important predictors are hierarchically identified, and potential cases are classified as PID cases or not at each node. In this analysis, potential predictors considered included ICD-9 codes (Table 1), age at diagnosis, whether treatment appropriate for PID was given, inpatient admission, whether chlamydia testing was conducted, and other concurrent diagnoses (described above). This process is repeated multiple times until the optimal tree is built. At each branch, data are optimally split to maximize the differentiation of observations based on the dependent variable; in this case, the dependent variable was a confirmed clinical PID diagnosis (yes/no) from medical record review.

The PID case-finding algorithm developed using GH data was then applied to the KPCO data. Algorithm performance was assessed by comparing the PID case status predicted by the algorithm to the PID case status determined by medical record review in each sample dataset (GH and KPCO). Summary statistics evaluating the performance of the algorithm were calculated in the sample population of women with ICD-9 codes related to PID; the medical record review results were assumed to be the truth. Sensitivity was defined as the proportion of PID cases correctly classified by ICD-9 code and confirmed by medical record review as PID cases that were identified as PID by the algorithm. Specificity was the proportion of PID cases identified by ICD-9 code but determined to not be PID that were correctly classified as not PID by the algorithm. Negative predictive value (NPV) was calculated as the proportion of potential cases classified by the algorithm as not PID that were identified by ICD-9 code and found not to be PID by medical record review. Positive predictive value (PPV) was defined as the proportion of algorithm-classified PID cases that were confirmed to be PID by medical record review. The PPV of selecting PID cases using ICD-9 codes alone was also calculated. The overall misclassification proportion was calculated as the proportion of potential PID cases incorrectly classified by the algorithm when compared to the medical record review findings. The 95% confidence intervals (CIs) based on the binomial distribution were calculated for all performance measures.

Analyses were conducted using SAS version 9.1.2 (SAS Institute Inc., Cary, NC), R (R Foundation for Statistical Computing, Vienna, Austria), and OpenEpi [20, 21]. The CART analysis was performed using "rpart" in the R package.

3. Results

Among the sample of 393 potential PID cases identified at GH using ICD-9 codes alone, 275 (70.0%) were confirmed to be clinical PID based on medical record review; 74 (18.8%) were not PID; 6 (1.5%) were of uncertain case status; 38 (9.7%) had no information available regarding the visit where the PID ICD-9 code was recorded (Table 2). Of the sample of 500 potential KPCO PID cases, 349 (69.8%) were confirmed to be PID, 92 (18.4%) were not PID, 5 (1.0%) were uncertain, and 54 (10.8%) had no information available on the visit.

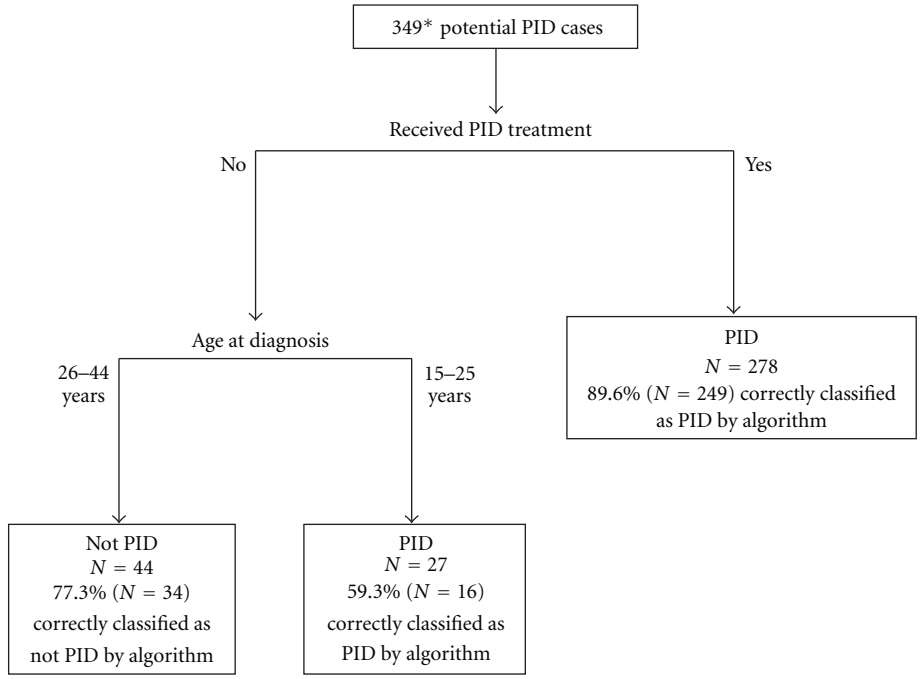


Figure 1: Case-finding algorithm developed using automated administrative data from Group Health Cooperative to refine identification of PID among a sample of women with ICD-9 codes suggestive of PID. PID: pelvic inflammatory disease. *Of 393 potential PID cases with ICD-9 codes associated with PID, 44 were not included due to uncertainty of PID case status after medical record review.

Of the 14 ICD-9 codes used to identify potential PID cases from GH, 614.9 was the most common, associated with 64.1% of the 393 potential cases (Table 1). The majority of visits where a potential PID case was identified had only one ICD-9 selected (92.4%), 5.9% had two codes, and 1.8% had three or more. In GH, 68.0% of the 275 confirmed PID cases had an ICD-9 code of 614.9. Of the 500 potential PID cases at KPCO, 50.4% were coded with the 614.9 ICD-9 code; 48.4% of the 441 confirmed PID cases had the 614.9 code recorded.

When using ICD-9 codes alone to identify PID cases at GH, the PPV was 78.8% (95% CI: 74.1–83.0%). The results were similar for KPCO, where the PPV for using ICD-9 codes was 79.1% (95% CI: 75.0–82.8).

The PID case-finding algorithm is shown in Figure 1. Of the 393 potential PID cases at GH, the 44 with uncertain case status or no information available were excluded. Thus, 349 potential PID cases were used to develop the algorithm. Two predictors of clinical PID were identified by the algorithm. The strongest predictor identified was the presence of treatment appropriate for PID. The algorithm classified 278 potential cases with documented treatment in administrative data as PID cases, of which 249 (89.6%) were confirmed as clinically diagnosed PID. Among those women with no PID treatment recorded, younger age was found to be the most important predictor. Specifically, young women between 15–25 years of age who had not received PID treatment were classified by the algorithm as PID cases. Among 27 such women, 16 (59.3%) were confirmed PID cases. Among 44 women who had no PID treatment and were aged 26–44 years, 34 (77.3%) were correctly classified by the algorithm as not having PID. No specific ICD-9 code was a stronger predictor than PID treatment and age.

The summary statistics of the algorithm performance using GH and KPCO sample data are shown in Table 3. At GH, algorithm sensitivity was 96.4% (95% CI: 93.4–98.2%), specificity was 45.9% (95% CI: 34.3–57.9%), NPV was 77.3% (95% CI: 62.2–88.5%), and PPV was 86.9% (95% CI: 82.9–90.5%). Using the algorithm, 14.3% of potential PID cases in the sample population of women with ICD-9 codes related to PID were misclassified. When applied to the validation dataset from KPCO, algorithm sensitivity was 90.3% (95% CI: 86.7%–93.2%), specificity was 37.0% (95% CI: 27.1%–47.7%), NPV was 50.0% (95% CI: 37.6%–62.4%), and PPV was 84.5% (95% CI: 80.4–88.0); 20.9% of the potential cases were misclassified.

The distribution of the two predictors included in the algorithm was similar for PID treatment between GH and KPCO potential PID cases, but different for age at PID diagnosis. In GH, 90.6% (249/275) of confirmed PID cases had documented treatment appropriate for PID, compared to 39.2% (29/74) of cases found not to be PID. Likewise, in KPCO, 84.0% (293/349) of confirmed PID cases had documented appropriate antimicrobial treatment, compared to 38.0% (35/92) of non-PID cases. When examining age at diagnosis in GH, 49.1% (135/275) of confirmed PID cases were <26 years, compared to 28.4% (21/74) of cases confirmed by medical record review to not be PID. However, in KPCO women aged <26 years accounted for 38.1% (133/349) of confirmed PID cases and 41.3% (38/92) of cases that were not PID.

Table 3: Performance statistics for PID case identification using an algorithm applied to sample administrative data from women with an ICD-9 code* related to PID.

(a) Accuracy of PID case-finding algorithm: GH development dataset.

		New algorithm classification		Total
		Not PID	PID	
Chart-confirmed Diagnosis	Not PID	34	40	74
	PID	10	265	275
	Total	44	305	349

(b) Accuracy of PID case-finding algorithm: KPCO validation dataset.

		New algorithm classification		Total
		Not PID	PID	
Chart-confirmed Diagnosis	Not PID	34	58	92
	PID	34	315	349
	Total	68	373	441

(c)

Performance Statistics (95% CI)	GH development dataset	KPCO validation dataset
PID case identification using ICD-9 codes* alone		
PPV	78.8% (74.1–83.0%)	79.1% (75.0–82.8%)
PID case identification using algorithm		
Sensitivity	96.4% (93.4–98.2%)	90.3% (86.7–93.2%)
Specificity	45.9% (34.3–57.9%)	37.0% (27.1–47.7%)
PPV	86.9% (82.6–90.5%)	84.5% (80.4–88.0%)
NPV	77.3% (62.2–88.5%)	50.0% (37.6–62.4%)
Proportion of potential cases misclassified	14.3% (10.8–18.5%)	20.9% (17.2–25.0%)

* ICD-9 codes shown in Table 1. Only potential cases with complete chart-review information are included.
GH: Group Health, KPCO: Kaiser Permanente Colorado, PID: pelvic inflammatory disease, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value.

4. Discussion

One of the primary goals in STD prevention is to reduce the burden of STD-associated infertility. Monitoring trends in PID, an intermediate adverse outcome between STD acquisition and the development of infertility, may help identify progress in STD prevention. However, surveillance of PID, which often relies on case identification from administrative data sources, has been historically difficult.

To identify clinical diagnoses of PID from medical records data for surveillance and research purposes, administrators and researchers have traditionally relied solely on ICD-9 diagnostic codes. In this study, using a standard set of ICD-9 codes to identify potential PID cases, a simple approach, the PPV relative to medical record review, was fairly high, about 79% at both sites. However, use of ICD-9 codes has limitations, including a lack of specificity [14], nonstandard application (especially when multiple codes may be used to designate a condition), and varying usage across individuals and healthcare sites in selecting which ICD-9 codes to use.

The algorithm developed in this analysis incorporated additional automated data elements as a practical alternative to medical record review to improve PID case finding among the subset of women with PID-related ICD-9 codes. Algorithm sensitivity (GH = 96.4%; KPCO = 90.3%) and PPV (GH = 86.9%; KPCO = 84.5%) were high at both sites, but higher at the site where the algorithm was developed (GH). However, specificity and NPV were low at both sites, although, again, performance was better at the algorithm development site. In GH, the proportion of potential PID cases misclassified by the algorithm was 14.3%; at KPCO, the proportion of cases that were misclassified using algorithm was 20.9%. Thus, the algorithm developed using GH data did not appear to perform as well in this second site. However, when using ICD-9 codes alone to identify potential PID cases, the PPV was 79%; the PPV increased to 85% in KPCO and 87% in GH when the algorithm was applied. Given the challenges in case identification and PID surveillance, small improvements such as the availability of a case-finding algorithm offer opportunities to move beyond the practice of identifying PID cases based only on ICD-9 codes. As this

study indicates, the extent of such value may be specific to the population under evaluation.

Currently, due to widespread data limitations, public health professionals must rely primarily on ecologic comparisons of STD incidence trends, PID diagnosis trends, and concurrent sexually transmitted disease (STD) prevention activities to evaluate programmatic impact. As data systems improve, ascertainment of STD-specific PID diagnoses may be possible with better automated linkages between laboratory data, clinical data, and inclusion of additional administrative data. The expanded use of electronic medical records will likely offer opportunities to further enhance surveillance of STD-associated PID. The identification of possible methods to improve PID case finding will be a contributing factor.

This analysis has several limitations. Due to budget and time constraints, only a limited number of medical record abstractions were possible and thus, the algorithm developed may not be robust. A larger sample may have resulted in a different algorithm. A random sample of potential PID cases based on ICD-9 diagnostic codes was selected in both sites, and the data used to develop and validate the algorithm should be generally representative of the entire population of potential PID cases with an associated ICD-9 code in GH and KPCO during the study period. In this analysis, potential PID cases were identified using ICD-9 codes; ICD-9 codes may not be applied consistently across healthcare settings. No information on the population with PID not identified by ICD-9 codes (false negatives) was available; therefore, the sensitivity, specificity, and negative predictive value of using ICD-9 codes alone to identify clinically diagnosed PID cases could not be determined. Lastly, the algorithm performance statistics did not include women whose clinical PID status was missing or unable to be determined; fortunately, this finding occurred in a fairly low proportion of potential cases. While the algorithm would classify these cases as PID or not, the performance of the algorithm could not be assessed without medical record review information.

Strengths of this analysis include an evaluation of the group of ICD-9 codes currently in use to identify PID in administrative data. This study is one of the first to examine how these codes perform relative to clinical PID case detection as defined by medical record review. The utilization of CART methodology represents another strength. This strategy allowed for a comprehensive evaluation of additional available automated predictors of clinically diagnosed PID cases and all possible value splits of those predictors without the necessity of making assumptions about underlying variable distributions (a nonparametric approach). Interpretation of the CART findings is straightforward and was easily applied to another external setting (KPCO) after initial algorithm development to allow for an assessment of algorithm robustness.

5. Conclusions

Monitoring PID is important in assessing STD prevention and control efforts, particularly prevention of chlamydia and gonorrhea. The approaches utilized in this analysis may help improve PID surveillance efforts. While the challenge of diagnosing PID remains, results in the two study settings show that the PPV of using ICD-9 codes alone to predict a clinical PID diagnosis was quite high. At GH, the PID case-finding algorithm also offers a practical alternative to further refine PID case identification among the group of women with ICD-9 codes suggestive of PID. Further exploration of case-findings predictors in a larger sample may result in a more robust and generalizable algorithm. Research on additional novel approaches to identify clinical PID cases from administrative data should continue.

Disclosure

The findings and conclusions in this report have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.

Acknowledgment

The authors wish to acknowledge and thank Dr. James Buehler at the Centers for Disease Control and Prevention (CDC), for providing valuable guidance on the framing of this paper.

References

[1] M. Y. Sutton, M. Sternberg, A. Zaidi, M. E. S. Louis, and L. E. Markowitz, "Trends in pelvic inflammatory disease hospital discharges and ambulatory visits, United States, 1985–2001," *Sexually Transmitted Diseases*, vol. 32, no. 12, pp. 778–784, 2005.

[2] J. Paavonen, L. Westrom, and D. Eschenbach, "Pelvic inflammatory disease," in *Sexually Transmitted Diseases*, K. K. Holmes, P. F. Sparling, W. E. Stamm et al., Eds., pp. 1529–1561, McGraw-Hill, 4th edition, 2008.

[3] C. L. Haggerty, S. L. Gottlieb, B. D. Taylor, N. Low, F. Xu, and R. B. Ness, "Risk of sequelae after *Chlamydia trachomatis* genital infection in women," *Journal of Infectious Diseases*, vol. 201, no. 2, pp. S134–S155, 2010.

[4] P. Oakeshott, S. Kerry, A. Aghaizu et al., "Randomised controlled trial of screening for *Chlamydia trachomatis* to prevent pelvic inflammatory disease: the POPI (prevention of pelvic infection) trial," *BMJ*, vol. 340, p. c1642, 2010.

[5] L. A. Wallace, A. Scoular, G. Hart, M. Reid, P. Wilson, and D. J. Goldberg, "What is the excess risk of infertility in women after genital chlamydia infection? A systematic review of the evidence," *Sexually Transmitted Infections*, vol. 84, no. 3, pp. 171–175, 2008.

[6] M. Macaluso, T. J. Wright-Schnapp, A. Chandra et al., "A public health focus on infertility prevention, detection, and management," *Fertility and Sterility*, vol. 93, no. 1, pp. 16.e1–16.e10, 2010.

[7] Centers for Disease Control and Prevention and American Society for Reproductive Medicine, *Assisted Reproductive Therapy Success Rates: National Summary and Fertility Clinic Reports*, Department of Health and Human Services, Atlanta, GA, USA, 2009.

[8] Centers for Disease Control and Prevention, "Sexually transmitted diseases treatment guidelines, 2010," *MMWR*, vol. 59, pp. 1–110, 2010.

[9] N. Calonge, D. B. Petitti, T. G. DeWitt et al., "Screening for chlamydial infection: U.S. preventive services task force recommendation statement," *Annals of Internal Medicine*, vol. 147, no. 2, pp. 128–134, 2007.

[10] L. Østergaard, B. Andersen, J. K. Møller, and F. Olesen, "Home sampling versus conventional swab sampling for screening of *Chlamydia trachomatis* in women: a cluster-randomized 1-year follow-up study," *Clinical Infectious Diseases*, vol. 31, no. 4, pp. 951–957, 2000.

[11] D. Scholes, A. Stergachis, F. E. Heidrich, H. Andrilla, K. K. Holmes, and W. E. Stamm, "Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection," *The New England Journal of Medicine*, vol. 334, no. 21, pp. 1362–1366, 1996.

[12] Centers for Disease Control and Prevention, "Sexually transmitted diseases treatment guidelines, 2006," *MMWR*, vol. 55, no. 7, pp. 38–40, 2006.

[13] I. Simms, F. Warburton, and L. Weström, "Diagnosis of pelvic inflammatory disease: time for a rethink," *Sexually Transmitted Infections*, vol. 79, no. 6, pp. 491–494, 2003.

[14] S. Ratelle, D. Yokoe, C. Blejan et al., "Predictive value of clinical diagnostic codes for the CDC case definition of pelvic inflammatory disease (PID): implications for surveillance," *Sexually Transmitted Diseases*, vol. 30, no. 11, pp. 866–870, 2003.

[15] M. K. Bohm, L. Newman, C. L. Satterwhite, G. Tao, and H. S. Weinstock, "Pelvic inflammatory disease among privately insured women, United States, 2001–2005," *Sexually Transmitted Diseases*, vol. 37, no. 3, pp. 131–136, 2010.

[16] D. Scholes, C. L. Satterwhite, O. Yu et al., "Long-term trends in *Chlamydia trachomatis* infections and relatedoutcomes in a U.S. managed care population," *Sexually Transmitted Diseases*. In press.

[17] D. Scholes, O. Yu, M. A. Raebel, B. Trabert, and V. L. Holt, "Improving automated case finding for ectopic pregnancy using a classification algorithm," *Human Reproduction*, vol. 26, no. 11, pp. 3163–3168, 2011.

[18] W. Lo-Ciganic, J. C. Zgibor, K. Ruppert, V. C. Arena, and R. A. Stone, "Identifying type 1 and type 2 diabetic cases using administrative data: a tree-structured model," *Journal of Diabetes Science and Technology*, vol. 5, no. 3, pp. 486–493, 2011.

[19] L. Breiman, J. Friedman, C. J. Stone et al., *Classification and Regression Trees*, Taylor and Francis, Belmont, Calif, USA, 1984.

[20] A. Dean, K. Sullivan, and M. Soe, "OpenEpi: open source epidemiologic statistics for public health, version 2.3.1," 2010, http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm.

[21] RDC Team, "R: a language and environment for statistical computing," Vienna, Austria: R Foundation for Statistical Computing, 2008, http://www.r-project.org.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 989762, 8 pages
doi:10.1155/2011/989762

Research Article

The Role of *Chlamydia trachomatis* Polymorphic Membrane Proteins in Inflammation and Sequelae among Women with Pelvic Inflammatory Disease

Brandie D. Taylor,[1,2] Toni Darville,[3] Chun Tan,[4] Patrik M. Bavoil,[4] Roberta B. Ness,[5] and Catherine L. Haggerty[1]

[1] *Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA*
[2] *Department of Epidemiology, Michigan State University, 644 West Fee Hall, East Lansing, MI 48824, USA*
[3] *Department of Pediatrics, University of Pittsburgh Medical Center, Pittsburgh, PA 15224, USA*
[4] *Department of Microbial Pathogenesis, University of Maryland Dental School, Baltimore, MD 21201, USA*
[5] *School of Public Health, The University of Texas Health Science Center, Houston, TX 77030, USA*

Correspondence should be addressed to Brandie D. Taylor, btaylor@epi.msu.edu

Received 2 June 2011; Revised 30 July 2011; Accepted 10 August 2011

Academic Editor: Thomas Cherpes

Chlamydia trachomatis polymorphic membrane proteins (Pmps) may increase genital tract inflammation and play a role in virulence. Antibody levels for PmpA, PmpD, and PmpI, measured in densitometric units, were assessed among a pilot sample of 40 *C. trachomatis*-infected women with mild-to-moderate clinical PID. Women who expressed antibodies to PmpA were less likely to achieve pregnancy (40.0% versus 85.7%; $P = 0.042$) and less likely to have a live birth (0.0% versus 80.0%; $P = 0.005$) compared to women who did not express antibody to PmpA. Women who expressed antibodies to PmpI were more likely to have upper genital tract infection (61.5% versus 20.0%; $P = 0.026$). However, seropositivity to PmpI and PmpD did not modify the risk of reproductive sequelae or inflammation. Seropositivity to chlamydial PmpA may represent a biomarker of increased risk of sequelae secondary to infection with *C. trachomatis*.

1. Introduction

Chlamydia trachomatis is the most common bacterial sexually transmitted infection in the United States [1]. In women, *C. trachomatis* can ascend from the endocervix to the upper genital tract and cause pelvic inflammatory disease (PID) and serious reproductive morbidity including infertility and ectopic pregnancy [2]. However, rates of progression vary and 80% or more of women with chlamydia do not develop PID [1]. Some women clear chlamydial infection without tissue damage, while in some cases *C. trachomatis* induces a chronic low-grade infection [3]. This may lead to persistent inflammation of the upper genital tract causing long-term reproductive sequelae. The pathogenesis of *C. trachomatis* disease is not well-understood, and pathogen-specific virulence factors that may contribute to variability in the course and outcome of infection have not been identified.

Nine surface-exposed *C. trachomatis* polymorphic membrane proteins (Pmps) are encoded via a multigene family yielding PmpA to PmpI [4]. Pmps represent 13.6% of the coding capacity of the *C. trachomatis* genome [4], suggesting they have a critical role in biology and virulence [5, 6]. However, the role of Pmps in chlamydial virulence is not well understood. PmpD is a species-common, pan-neutralizing antigen hypothesized to hold potential as a vaccine candidate [6]. Thus, the development of high titers of antibody to PmpD might protect from infection or disease. On the other hand, *Chlamydia pneumoniae* Pmps have been shown to induce proinflammatory mediators in infected host cells, demonstrating the potential for these proteins to play a direct role in pathogenesis [7, 8]. All nine Pmps are expressed on the surface of chlamydial elementary bodies (EB) and *C. trachomatis*-infected patients can produce antibodies to each Pmp subtype [9]. However, antibody profiles vary among

C. trachomatis-infected patients [5]. In addition, comparative genomics has revealed genetic variation and rearrangements among *pmp* gene families in different strains and isolates [5, 9]. This suggests that immune pressure leads to antigenic variation in these surface-exposed proteins [5, 9], a further indication that these proteins have a role in chlamydial virulence.

Pmps may be involved in virulence, but very little is known about their role in the development of PID and adverse reproductive sequelae. Tan et al. examined variation in Pmp-specific antibody responses in four distinct patient populations, demonstrating that women with PID had significantly higher reactivity to PmpB and PmpI compared to adolescent females with lower genital tract infection [5]. These data may reflect a role for these specific Pmps in inflammation, or simply that women with PID had sustained increased exposure due to repeated or chronic infection.

In a separate study, Tan et al. found that Pmps exhibit on/off switching *in vitro* which enables independent expression of each Pmp [9]. PmpA, PmpD, and PmpI had very low "off" frequencies of 0.5–1%, suggesting that expression of these Pmps provides an *in vitro* phenotypic advantage [9]. This may or may not translate into enhanced *in vivo* virulence. The high "on" frequencies of PmpD and PmpI correlate with the fact that anti-PmpD and -PmpI antibodies are commonly detected in *C. trachomatis*-infected patients. However, despite the high "on" frequency of PmpA, anti-PmpA antibodies are relatively rare. Further research from this group found that Pmp transcriptional units are differentially expressed during chlamydial development [10]. In addition, Pmp expression was altered under penicillin-induced stress, except for the expression of PmpA, PmpD, and PmpI, which remained steady [10]. Taken together, these data suggest an importance for PmpA, PmpD, and PmpI in chlamydial pathogenesis. However, the role of these Pmps in chlamydial PID has never been examined in humans. In order to examine the role of PmpA, PmpD, and PmpI in chlamydial pathogenesis, we conducted a pilot study to determine if antibody responses specific for these Pmps were associated with parameters of inflammation or sequelae in a group of women with documented *C. trachomatis* PID.

2. Methods

This study utilized data from the PID evaluation and clinical health (PEACH) study. This was the first randomized clinical trial to compare inpatient and outpatient treatment in preventing long-term complications among 831 women with mild-to-moderate PID. The methods of subject recruitment, data collection, and followup have been reported elsewhere [11]. Briefly, between March 1996 and February 1999, women aged 14–37 years were recruited from emergency departments, obstetrics and gynecology clinics, sexually transmitted disease clinics, and private practices at 7 primary and 6 secondary sites throughout the eastern, southern, and central regions of the United States. Women who had suspected PID and gave informed consent were eligible for the PEACH study. Women were enrolled on the basis of clinically generalizable criteria for suspected PID. Eligibility included a history of pelvic discomfort for less than 30 days, findings of pelvic organ tenderness (uterine or adnexal) on bimanual examination, and leukorrhea and/or mucopurulent cervicitis and/or untreated but documented gonococcal or chlamydial cervicitis. The University of Pittsburgh Institutional Review Board approved the study.

A total of 2941 women were screened for study entry, of those 346 (11.8%) did not meet the clinical inclusion criteria for randomization. Women were additionally excluded if they were pregnant ($n = 141$, 4.8%); had taken antimicrobials within the past 7 days ($n = 248$, 8.4%); had a history of hysterectomy or bilateral salpingectomy ($n = 248$, 8.4%); had an abortion, delivery, or gynecologic surgery within the past 14 days ($n = 51$, 1.7%); had a suspected tubo-ovarian abscess or other condition requiring surgery ($n = 191$, 6.5%); had an allergy to the study medications ($n = 163$, 5.5%); were homeless ($n = 29$, 1%); or had vomiting after a trial of antiemetic treatment ($n = 11$, 0.4%). A total of 831 were enrolled and were contacted at least once after randomization. Our analysis included a pilot sample of 40 *C. trachomatis*-positive women, whose serum samples were previously analyzed for Pmp antibodies [5]. All sera were collected at baseline.

Women were randomized to either inpatient treatment of intravenous cefoxitin every 6 hours and doxycycline orally twice a day for 14 days; or outpatient treatment consisting of a single intramuscular injection of cefoxitin and oral doxycycline twice a day for 14 days. Because the treatment modality was not associated with reproductive morbidities in the PEACH study [12], we do not include them as a covariate in this analysis. Participants were followed-up with in-person visits at 5 and 30 days after treatment. At the 30-day followup, the gynecological exam was repeated. Telephone followups were conducted by the study nurses every 3 months during the first year after enrollment and then every 4 months until June 2004. At that point, information was obtained by self-report for 69.1% of the cohort, with a mean followup of 84 months.

A pelvic examination and interview were conducted at the baseline visit. The interview collected information on reason for visit, brief pain history, demographics, history of PID/sexually transmitted diseases, sexual and contraceptive history, reproductive decisions, douching history, pregnancy history, medical and gynecological history, and lifestyle habits. Followup interviews collected self-reported information on pelvic pain, pregnancy and births, signs and symptoms of PID, STDs, contraceptive use, pattern of sexual intercourse, and health care utilization.

Gynecological examinations were performed at baseline and 5 and 30 days after treatment. Endometrial biopsy and cervical swab specimens were obtained for histological examination including chlamydial polymerase chain reaction (PCR) and gonococcal culture. All cultures and PCR were performed at a central reference laboratory. For the patients with endometrial biopsies, two reference pathologists separately evaluated at least one section stained with hematoxylin and eosin and at least one stained with methyl green pyronin. A disagreement about the presence or absence of neutrophils and plasma cells was settled by both pathologists reading the slides together and coming

to an agreement. Histological endometritis was based on a modification of the criteria proposed by Kiviat et al. [13]. Endometritis was defined as the presence of at least five neutrophils in the endometrial surface epithelium in the absence of menstrual endometrium and/or at least two plasma cells in the endometrial stroma. This definition has been found to be the best predictor of upper genital tract infection plus salpingitis, with a sensitivity of 92% and specificity of 87% [13].

Reproductive outcomes were assessed over a mean of 84 months. Measures of fertility included infertility, live birth, and pregnancy. Other reproductive outcomes included recurrent PID and chronic pelvic pain. Infertility was determined among women reporting no birth control or methods considered being unreliable, including withdrawal, rhythm method, vasectomy, or using the following methods rarely or occasionally:diaphragm, condoms, spermicidal foam/cream/jelly/suppositories, or cervical cap. Infertility was defined by lack of conception despite unprotected intercourse during 12 or more months of followup. Self-reported pregnancy was determined by a positive urine/blood test or physician's diagnosis among all women in the cohort. Live birth was determined among all women in the cohort by self-report during followup. Recurrent PID was self-reported and verified whenever medical records were available (45% of cohort). Women were considered to have recurrent PID if they experienced a subsequent episode of PID more than 30 days after the index illness. Chronic pelvic pain was defined by two or more consecutive reports of pelvic pain during telephone followup interviews administered through 32 months [14]. This translates to approximately 6 months or greater duration of pain [14]. Data from at least 2 followup visits were required to determine chronic pelvic pain.

Pmp antibody levels were previously measured among a subset of 40 *C. trachomatis*-positive women who had stored serum samples available. Pmp antibody levels were measured in densitometric units. These methods have been described elsewhere [5]. Briefly, purified EBs or partially purified rPmp polypeptides (rPmpD-FL and rPmpI-N) were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Amounts of rPmps were standardized by immunoblot analysis with anti-His tag antibody (1 : 1,500; GE Healthcare). Blots were visualized using Molecular Dynamics Typhoon 9400 imager (Amersham Biosciences, NJ). Antibody reactivity against the highest molecular-mass band in each lane was analyzed using Image Quant 5.2 image analysis software (Molecular Dynamics Sunnyvale, Calif). Serum response against each rPmp was quantified by the volume of the band. Data were normalized against His-tag-specific antibody reactivity.

Since PmpA, PmpD, and PmpI are uniquely expressed and may play a role in chlamydial pathogenesis [9, 10], we chose to only include these Pmps in our analyses. Our objective was to determine if markers of inflammation or sequelae differed between women who displayed antibody reactivity to PmpA, PmpD, or PmpI and those who did not. Both continuous and binary variables were used. Differences in baseline characteristics were compared between groups using Chi-square or Fisher's exact tests and t-test for the normally distributed continuous variables. Chi-square or Fisher's exact tests were also used to compare differences in the frequency of inflammatory markers (elevated white blood cell count (WBC), temperature, elevated C-reactive protein (CRP), bilateral adnexal tenderness, cervicitis, endometritis, upper genital tract infection (UGTI), erythrocyte sedimentation rate (ESR)), and reproductive outcomes (infertility, pregnancy, live birth, chronic pelvic pain, and recurrent PID) between groups. We also examined these relationships using a continuous variable for Pmp antibody response. As the distributions of PmpA, PmpD, and PmpI were skewed, nonparametric tests were used.

We also sought to determine if high levels of Pmp antibody expression were associated with inflammation or sequelae. After running sensitivity analyses, antibody reactivity groups for PmpD and PmpI were defined using median cut-points (high reactivity: PmpD $\geq$ 0.41; PmpI $\geq$ 0.89 and low reactivity: PmpD $<$ 0.41; PmpI $<$ 0.89). Due to the small number of women who expressed antibodies to PmpA, we were unable to examine levels of antibody expression ($n = 5$). Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Cox regression was used to calculate hazard ratios and 95% CI for time-to-pregnancy and time-to-recurrent PID. Models were adjusted for age and race. Additionally, time to pregnancy was adjusted for history of infertility, which was self-reported at baseline. If any model had less than 5 in any cell, it was excluded from regression analysis. All analyses were completed using SAS V9.2 (Cary, NC).

3. Results

Overall, women in this cohort tended to be less than 25 years of age (85.0%), African American (77.5%), single (86.8%), and having at least a high school education (60.0%). At baseline, the majority of women reported abnormal vaginal discharge (62.5%), had bilateral adnexal tenderness (80.0%) and mucopurulent cervicitis (65.7%), and had chlamydia isolated from the cervix only (58.3%). Women who expressed antibody to PmpI were more likely to smoke compared to women who did not express PmpI antibody (56.7% versus 20.0%; $P = 0.0411$) (Table 1). There were no other significant differences in important baseline characteristics between women who expressed antibody to PmpA, PmpD, or PmpI and women who did not.

Results show that compared to women who did not express antibody to PmpA, rates of elevated WBC (40.0% versus 23.5%), increased CRP (66.7% versus 46.2%), increased ESR (40.0% versus 31.4%), endometritis (100% versus 60.7%), and UGTI (75.0% versus 46.8%) were higher among women who expressed antibody to PmpA (Table 2). However, these differences did not reach statistical significance. Similarly, there were no significant differences in the frequency of infertility, recurrent PID, or chronic pelvic pain between groups. However, only 40% of women with antibody reactivity to PmpA achieved pregnancy compared to 85.7% of women who did not express antibody reactivity to PmpA ($P = 0.042$). In addition, none of the women with antibody reactivity to PmpA had a live birth, while

Table 1: Baseline characteristics of women by Pmp antibody expression.

Characteristics	PmpA			PmpD			PmpI		
	No $n = 35$	Yes $n = 5$	P value	No $n = 10$	Yes $n = 30$	P value	No $n = 10$	Yes $n = 30$	P value
Demographics									
Age									
<25 years	30 (85.7)	4 (80.0)	0.7378	8 (80.0)	26 (86.7)	0.3213	8 (80.0)	26 (86.7)	0.3213
Race/ethnicity African American	27 (77.1)	4 (80.0)	0.8862	8 (80.0)	26 (76.7)	0.3350	8 (80.0)	23 (76.7)	0.3350
Married	4 (12.1)	1 (20.0)	0.6272	1 (10.0)	4 (14.3)	0.4079	1 (10.0)	4 (14.3)	0.4079
Uninsured	11 (33.3)	3 (60.0)	0.0876	6 (60.0)	18 (64.3)	0.2850	6 (60.0)	18 (64.3)	0.2850
Education less than high school	14 (40.0)	2 (40.0)	1.000	2 (20.0)	14 (87.5)	0.1091	2 (20.0)	14 (46.7)	0.1091
Clinical									
Neisseria gonorrhoeae	8 (25.8)	2 (50.0)	0.3134	3 (37.5)	7 (25.9)	0.2709	2 (22.2)	8(30.8)	0.3064
Mycoplasma genitalium	3 (12.5)	1 (25.0)	0.3954	1 (25.0)	3 (12.5)	0.3954	1 (16.7)	3 (13.6)	0.4513
Bacterial vaginosis	21 (67.7)	1 (20.0)	0.0584	5 (55.6)	17 (62.9)	0.2800	5 (55.6)	17 (63.0)	0.2800
History of PID	10 (28.6)	1 (20.0)	0.3970	4 (40.0)	7 (23.3)	0.1849	3 (30.0)	8 (26.7)	0.3038
History of chlamydia	15 (45.5)	3 (60.0)	0.3089	4 (44.4)	14 (48.3)	0.7183	5 (55.6)	13 (44.8)	0.4272
[a]Pelvic pain (mean score ± SD)	65 ± 22	75 ± 15	0.3605	69 ± 14	66 ± 23	0.7387	65 ± 16	67 ± 17	0.7810
[b]Days of pain(mean score ± SD)	8 ± 7	5 ± 2	0.3238	6 ± 6	9 ± 7	0.3637	7 ± 7	8 ± 7	0.7569
Behavior									
Current smoker	15 (42.9)	4 (80.0)	0.1237	4 (40.0)	5 (50.0)	0.2481	2 (20.0)	17 (56.7)	0.0411
Drug use	12 (34.3)	2 (40.0)	0.3596	4 (40.0)	10 (33.3)	0.2719	4 (40.0)	10 (33.3)	0.2719

[a] (mean of current pelvic pain score, average pelvic pain score and worst pelvic pain score) × 10; [b]Self-reported time to treatment following onset of symptoms.

80% of women without antibody reactivity to PmpA had a live birth ($P = 0.005$). When examined as a continuous variable the results did not differ. Expression of anti-PmpA antibody was significantly increased in women who did not achieve pregnancy ($P = 0.0192$) or did not achieve a live birth ($P = 0.0043$).

There were no significant differences in inflammatory markers or reproductive sequelae between women who displayed antibody reactivity to PmpD and women who did not (Table 3). Results did not change when an antibody response to PmpD was considered as a continuous variable. Results were similar for PmpI (Table 4). However, women expressing antibody to PmpI were more likely to have UGTI compared to women who did not express antibody to PmpI (61.5% versus 20.0%; $P = 0.026$). Women who expressed antibody to PmpI were slightly more likely to have endometritis (72.7% versus 50.0%) although this did not reach statistical significance ($P = 0.2096$). When PmpI antibody response was examined as a continuous variable, the mean expression did not significantly differ between those with UGTI and those without UGTI ($P = 0.276$).

When levels of antibody expression for PmpD and PmpI were examined, there were no significant differences in the frequency of inflammatory markers or reproductive sequelae between high and low antibody reactivity groups. Although nonsignificant, women with high PmpD reactivity were slightly more likely to have an elevated WBC count (33.3% versus 16.7%), elevated ESR (42.9% versus 21.1%), elevated CRP (62.5% versus 37.5%), mucopurulent cervicitis (77.8% versus 52.9%), and endometritis (75.0% versus 56.3%) compared to women with low PmpD reactivity. Women with high PmpI reactivity were slightly more likely to have elevated CRP (55.6% versus 42.9%), bilateral adnexal tenderness (85.0% versus 75.0%), mucopurulent cervicitis (72.2% versus 58.8%), endometritis (71.4% versus 61.1%), and UGTI (58.8% versus 42.1%) compared to women with low PmpI reactivity although this did not reach statistical significance. Logistic regression also revealed no significant associations.

Similarly, a nonsignificant decrease in pregnancy rates (adjusted hazard ratio (AHR) 0.7, 95% CI 0.3–1.6) and increase in recurrent PID were observed for women with high PmpD antibody reactivity (AHR 1.3, 95% CI 0.2–8.3) (Table 5). In contrast, high PmpI antibody reactivity had minimal effects on sequelae. However, after adjustments, women with high PmpI antibody reactivity showed a nonsignificant trend towards decreased live births (AOR 0.6, 95% CI 0.1–4.0).

4. Discussion

Among women with mild-to-moderate chlamydial PID, those who expressed antibody to PmpA were less likely to achieve pregnancy and less likely to report a live birth. The overall effects of seropositivity for PmpD and PmpI on inflammation and reproductive sequelae were minimal. However, women who expressed PmpI antibody were more likely to have UGTI. Trends towards elevated baseline genital

Table 2: Frequency of baseline inflammatory markers and reproductive sequelae by PmpA antibody expression.

Inflammation and sequelae	PmpA		*P* value
	No $n = 35$	Yes $n = 5$	
Inflammation			
[a]Elevated temperature (>100.4°F)	0 (0.0)	1 (25.0)	0.1111
[a]Elevated WBC count (>10,000 mm^3)	8 (23.5)	2 (40.0)	0.2856
Erythrocyte sedimentation rate (>15 mm/hr)	11 (31.4)	2 (40.0)	0.7019
[a]C-reactive protein (>5 mg/dL)	6 (46.2)	2 (66.7)	0.5218
Bilateral adnexal tenderness	28 (80.0)	4 (80.0)	1.000
[a]Mucopurulent cervicitis	20 (66.7)	3 (60.0)	0.7712
Upper genital tract infection	15 (46.7)	3 (75.0)	0.2494
[a]Endometritis	17 (60.7)	4 (100.0)	0.1664
Reproductive sequelae			
Infertility	5 (14.3)	0 (0.0)	0.3663
[b]Live birth	20 (80.0)	0 (0.0)	0.0053
Pregnancy	30 (85.7)	1 (40.0)	0.0422
[b]Chronic pelvic pain	13 (38.2)	0 (0.0)	0.1142
[b]Recurrent PID	6 (17.7)	0 (0.0)	0.4122

[a]WBC data was available for 39 patients, CRP data was available for 16 patients, mucopurulent cervicitis data was available for 35 patients, UGTI data was available for 36 patients, and endometritis data was available for 32 patients; [b]live birth data was available for 29 patients; chronic pelvic pain and recurrent PID data were available for 39 patients.

Table 3: Frequency of baseline inflammatory markers and reproductive sequelae by PmpD antibody expression.

Inflammation and sequelae	PmpD		*P* value
	No $n = 10$	Yes $n = 30$	
Inflammation			
[a]Elevated temperature (>100.4°F)	0 (0.0)	1 (3.7)	0.7500
[a]Elevated WBC count (>10,000 mm^3)	2 (20.0)	8 (27.6)	0.3038
Erythrocyte sedimentation rate (>15 mm/hr)	3 (30.0)	10 (33.3)	0.2996
[a]C-reactive protein (>5 mg/dL)	2 (40.0)	6 (54.6)	0.3590
Bilateral adnexal tenderness	7 (70.0)	25 (83.3)	0.2224
[a]Mucopurulent cervicitis	4 (50.0)	19 (70.4)	0.1862
Upper genital tract infection	4 (50.0)	17 (70.8)	0.1878
[a]Endometritis	3 (33.3)	15 (55.6)	0.1609
Reproductive sequelae			
Infertility	1 (10.0)	4 (13.3)	0.4165
[b]Live birth	7 (77.8)	13 (65.0)	0.2787
Pregnancy	8 (80.0)	24 (80.0)	1.000
[b]Chronic pelvic pain	4 (44.4)	9 (30.0)	0.2219
[b]Recurrent PID	2 (22.2)	4 (13.3)	0.3024

[a]WBC data was available for 39 patients, CRP data was available for 16 patients, mucopurulent cervicitis data was available for 35 patients, UGTI data was available for 36 patients, and endometritis data was available for 32 patients; [b] live birth data was available for 29 patients; chronic pelvic pain and recurrent PID data were available for 39 patients.

tract and systemic inflammation, increased reproductive morbidity, and decreased pregnancy rates were observed among women with high PmpD antibody reactivity. Similarly, women with high PmpI antibody reactivity displayed trends towards elevated baseline inflammation. However, these results were nonsignificant.

The progression to PID following lower genital *C. trachomatis* infection varies. Among high-risk groups, 2–4.5% of women with untreated chlamydial infection will develop PID within 2 weeks, and 19% with treated chlamydial infection will develop PID within 3 years [1, 15–18]. Chlamydial virulence proteins may explain

Table 4: Frequency of baseline inflammatory markers and reproductive sequelae by PmpI antibody expression.

Inflammation and sequelae	PmpI		P value
	No $n = 10$	Yes $n = 30$	
Inflammation			
[a]Elevated temperature (>100.4°F)	0 (0.0)	1 (3.7)	0.7500
[a]Elevated WBC count (>10,000 mm^3)	2 (20.0)	8 (27.6)	0.3038
Erythrocyte sedimentation rate (>15 mm/hr)	3 (30.0)	10 (33.3)	0.2996
[a]C-reactive protein (>5 mg/dL)	2 (66.7)	6 (46.2)	0.4000
Bilateral adnexal tenderness	6 (60.0)	26 (86.7)	0.0748
[a]Mucopurulent cervicitis	5 (62.5)	18 (66.7)	0.3145
Upper genital tract infection	2 (20.0)	16 (61.5)	0.0263
[a]Endometritis	5 (50.0)	16 (72.7)	0.1457
Reproductive sequelae			
Infertility	1 (10.0)	4 (13.3)	0.4165
[b]Live birth	6 (75.0)	14 (66.7)	0.3251
Pregnancy	8 (80.0)	24 (80.0)	1.000
[b]Chronic pelvic pain	2 (20.0)	11 (37.9)	0.2996
[b]Recurrent PID	3 (30.0)	3 (10.3)	0.1344

[a]WBC data was available for 39 patients, CRP data was available for 16 patients, mucopurulent cervicitis data was available for 35 patients, UGTI data was available for 36 patients, and endometritis data was available for 32 patients; [b] live birth data was available for 29 patients; chronic pelvic pain and recurrent PID data were available for 39 patients.

Table 5: Effect of Pmp antibody reactivity on time-to-pregnancy and time-to-recurrent PID.

Subgroup	Pregnancy		Recurrent PID	
	Crude HR (95% CI)	[a]Adjusted HR (95% CI)	Crude HR (95% CI)	[a]Adjusted HR (95% CI)
[b]*High PmpD* ($n = 28$)	0.7 (0.3–1.3)	0.7 (0.3–1.6)	0.9 (0.2–4.7)	1.3 (0.2–8.3)
[c]*High PmpI* ($n = 23$)	1.3 (0.7–2.7)	1.4 (0.7–3.0)	0.6 (0.1–3.0)	0.7 (0.1–5.3)

[a]Models were adjusted for age, race, and models predicting infertility, pregnancy, and live birth were additionally adjusted for infertility self-reported at baseline; [b]PmpD antibody reactivity is based on a median cut-point; low reactivity <0.41, high reactivity ≥0.41; [c]PmpI antibody reactivity is based on a median cut-point; low reactivity <0.89, high reactivity ≥0.89.

differences in PID progression. As PmpA, PmpD, and PmpI have relatively low "off" frequencies (0.5–1%) [9], they should be present for antigenic processing and presentation allowing for antibody induction in the majority of infected individuals. In fact, PmpD and PmpI antibodies were frequent in our cohort. However, the prevalence of PmpA-expressing inclusions in *in vitro*-grown *C. trachomatis* does not correlate with the the low frequency of PmpA antibodies detected in our cohort of women with clinical PID and in other populations of *C. trachomatis*-infected patients [5]. The reason for this discrepancy is not entirely clear. Tan et al. suggest that Pmp expression in *in vitro*-grown *C. trachomatis* differs from *C. trachomatis* in the human genital tract [9]. Chlamydiae processing and secretion of Pmp fragments may also differ, possibly resulting in varied antibody expression. It is also possible that PmpA may have poor immunogenicity or that PmpA antibodies were generated at low levels not recognized by the initial SDS-PAGE analysis. Still, PmpA, PmpD, and PmpI are the most conserved Pmps of *C. trachomatis*, and their expression is unaltered in response to stress [10]. This may suggest that PmpA, PmpD, and PmpI are important for chlamydial survival and may play a role in chlamydial pathogenesis [9, 10].

Not all women with PID go on to develop reproductive morbidity. Studies have found a link between tubal occlusion and chlamydial antibodies [1, 19], as well as chlamydial infection and post-PID infertility [1, 20]. Inflammation caused by chlamydial infection may play a role in the development of infertility, through damage to the cilia lining of the Fallopian tubes, Fallopian tube blockage or closure, or adhesion formation among pelvic organs [2]. Our data indicate that women who express PmpA antibody were less likely to achieve pregnancy and less likely to have a live birth. Pregnancy and live birth can be used as markers of fertility. These variables are easier to define compared to infertility which requires classification of contraception than can be hampered by changing variables over time, concurrent use of more than 1 method of contraception, missing data, and compliance. Therefore, our results may suggest that PmpA plays a role in upper genital tract pathology. Although rates of inflammatory markers were increased among women who express PmpA antibody, no

statistically significant differences were found between the groups. These null findings could be a result of our limited power. Due to the low frequency of PmpA in our cohort, we were also unable to examine levels of antibody titer.

Data suggest that PmpD acts as an adhesion molecule and stimulates proinflammatory cytokines through the nuclear factor-κB pathway [7, 8]. Since PmpD may stimulate host cell inflammatory responses, it is possible that increased antibody to PmpD reflects increased exposure to these potentially pathogenic ligands. We did find increased inflammation and reproductive sequelae among women with high antibody titers to PmpD. However, these results were nonsignificant. Overall, expression of PmpD antibody appeared to have minimal effects on inflammation and reproductive sequelae in this study. Crane et al. reported that anti-PmpD antibodies result in neutralization of chlamydial elementary bodies and reduced infectivity *in vitro* [6]. In our *in vivo* study, we found no evidence for protection from disease in women with high seropositivity to PmpD. However, it should be noted that as our study used recombinant denatured material for the measurement of seroreactivity, *in vivo* antibody reactivity to native PmpD present on chlamydial elementary bodies may not be fully reflected.

Tan et al. found that the PEACH PID population had significantly higher levels of PmpI antibodies compared to adolescent females with lower genital tract infection ($P < 0.0001$) [5]. This could suggest that high titers to PmpI could be associated with chlamydial progression to the upper genital tract [10]. We did find that women with antibody reactivity to PmpI were more likely to have UGTI. Endometritis was also more frequent in this group although results were nonsignificant. When we examined PmpI as a continuous variable, we did not find a significant difference in PmpI antibody expression between women with UGTI and women without UGTI. Therefore, this finding may have been due to chance. Women from the PEACH cohort are older and have likely been exposed to *C. trachomatis* more often than adolescents. Screening studies have found that older women have less infection but increased chlamydial antibodies compared to adolescents [3]. Therefore, high antibody reactivity to Pmps could represent a measure of cumulative chlamydial exposure, indicating that women with more infections suffer greater sequelae. In fact, a study among 443 PEACH participants found that PID recurrence was higher (HR 2.48, 95% CI 1.00–6.27), and pregnancy rates were significantly lower (HR 0.47, 95% CI 0.28–0.79) among women whose antibody titers to chlamydia EB were in the highest tertile [21]. However, we were unable to find any significant associations between high antibody reactivity to PmpI or PmpD and reproductive sequelae. Further, we found no significant associations with markers of inflammation.

There could be several reasons for our mostly null findings. All women in our cohort had clinically suspected PID. Therefore, we were unable to compare women with chlamydial PID to women with uncomplicated *C. trachomatis* infection. Future studies should compare these groups to determine the role of Pmps in *C. trachomatis* progression. In addition, our sample size limited our power to detect significant associations. We must also consider other factors that may play a role in chlamydial pathogenesis. It is possible that host susceptibility may explain why some women with chlamydia experience sequelae and others do not. In fact, chlamydia is suggested to be a disease of immunopathology [3]. Therefore, genetic variations in host immune receptors may cause unfavorable inflammation and explain the variability in outcomes. Chlamydial load may also play a role in the course and outcome of infection. We did find a borderline association with cervicitis among women with high PmpD reactivity using logistic regression, and chlamydial cervicitis has been associated with a higher chlamydial load [22].

To our knowledge, this is the first study to examine the role of Pmp antibody response in inflammation and post-PID sequelae. Data were obtained from a prospective randomized clinical trial with comprehensive demographic, clinical, and obstetric measurements. Further, our findings are generalizable to patients treated for clinically suspected PID. However, as some patients with clinically suspected PID might actually have ovarian cysts, pelvic adhesions, or endometriosis [23], some women in our study may not have had true upper genital tract infection. We attempted to minimize some of this misclassification by excluding women reporting greater than 30 days of pain at the time of enrollment. We recognize that reproductive outcomes were based on self-reported data and that misclassification bias is possible. Our analysis of Pmp seropositivity is semiquantitative and cannot generate actual titers. However, it is still sufficient for comparative rough estimates of Pmp-subtype-specific titers. In addition, antibody expression may differ depending on the time course of the infection. We are unable to confirm when women first became infected with *C. trachomatis*. All women were recruited when they presented for care for symptoms. We do know that time to treatment does differ among women with PID [24]. Time to treatment did not significantly differ between any of our groups. Serovars for *C. trachomatis* were not determined in the PEACH study. However, PmpA, PmpD, and PmpI are the most conserved Pmps of *C. trachomatis*, and it is possible that their functions are also conserved across serovars [9, 10]. As this pilot study is limited by power, larger studies should continue to explore the role of Pmps in the course and outcome of *C. trachomatis* infection. Specifically, the correlation between PmpA antibody reactivity and reproductive sequelae needs to be confirmed.

Variability in the progression of chlamydial infection may be due to varied expression of chlamydial Pmps that are reflected by the serum anti-Pmp antibody response. Our data suggest that women who express PmpA antibody had decreased pregnancy rates and decreased live births. Rates of inflammatory markers were increased among women with PmpA antibody although these results were nonsignificant. In contrast, a positive antibody response to PmpD or PmpI appeared to relate minimally to reproductive sequelae and inflammation. Results were the same when high antibody reactivity to both PmpD and PmpI was explored. These results suggest a possible role for PmpA, but not for PmpD or PmpI, in upper genital tract pathology.

References

[1] C. Haggerty, S. Gottlieb, B. Taylor, N. Low, F. Xu, and R. Ness, "Risk of sequelae after Chlamydia trachomatis genital infection in women," *Journal of Infectious Diseases*, vol. 201, supplement 2, pp. 134–155, 2010.

[2] C. Haggerty and R. Ness, "Epidemiology, pathogenesis and treatment of pelvic inflammatory disease," *Expert Review of Anti-Infective Therapy*, vol. 4, no. 2, pp. 235–247, 2006.

[3] J. Schachter and R. Stephens, "Biology of *Chlamydia trachomatis*," in *Sexually Transmitted Diseases*, K. Homes, P. Sparling, W. Stamm et al., Eds., pp. 555–574, McGraw Hill, New York, NY, USA, 2008.

[4] R. Stephens, S. Kalman, C. Lammel et al., "Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*," *Science*, vol. 282, no. 5389, pp. 754–759, 1998.

[5] C. Tan, R. Hsia, H. Shou et al., "*Chlamydia trachomatis*-infected patients display variable antibody profiles against the nine-member polymorphic membrane protein family," *Infection and Immunity*, vol. 77, no. 8, pp. 3218–3226, 2009.

[6] D. Crane, J. Carlson, E. Fischer et al., "*Chlamydia tracomatis* polymorphic membrane protein D is a species-common pan-neutralizing antigen," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 6, pp. 1894–1899, 2006.

[7] W. Wehrl, V. Brinkmann, P. Jungblut, T. Meyer, and A. Szczepek, "From the inside out—processing of the Chlamydial autotransporter PmpD and its role in bacterial adhesion and activation of human host cells," *Molecular Microbiology*, vol. 51, no. 2, pp. 319–334, 2004.

[8] A. Niessner, C. Kaun, G. Zorn et al., "Polymorphic membrane protein (PMP) 20 and PMP 21 of *Chlamydia pneumoniae* induce proinflammatory mediators in human endothelial cells in vitro by activation of the nuclear factor-κB pathway," *Journal of Infectious Diseases*, vol. 188, no. 1, pp. 108–113, 2003.

[9] C. Tan, R. Hsia, H. Shou, J. Carrasco, R. Rank, and P. Bavoil, "Variable expression of surface-exposed polymorphic membrane proteins in *in vitro*-grown *Chlamydia trachomatis*," *Cellular Microbiology*, vol. 12, no. 2, pp. 174–187, 2010.

[10] J. Carrasco, C. Tan, R. Rank, R. Hsia, and P. Bavoil, "Altered developmental expression of polymorphic membrane proteins in penicillin-stressed *Chlamydia trachomatis*," *Cellular Microbiology*, vol. 13, no. 7, pp. 1014–1025, 2011.

[11] R. Ness, D. Soper, J. Peipert et al., "Design of the PID Evaluation and Clinical Health (PEACH) study," *Controlled Clinical Trials*, vol. 19, no. 5, pp. 499–514, 1998.

[12] R. Ness, D. Soper, R. Holley et al., "Effectiveness of inpatient and outpatient treatment strategies for women with pelvic inflammatory disease: results from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) Randomized Trial," *American Journal of Obstetrics and Gynecology*, vol. 186, no. 5, pp. 929–937, 2002.

[13] N. Kiviat, P. Wølner-Hanssen, D. Eschenbach et al., "Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis," *The American Journal of Surgical Pathology*, vol. 14, no. 2, pp. 167–175, 1990.

[14] C. Haggerty, R. Schulz, and R. Ness, "Lower quality of life among women with chronic pelvic pain after pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 102, no. 5, pp. 934–939, 2003.

[15] R. Ness, K. Smith, C. Chang, E. F. Schisterman, and D. C. Bass, "Prediction of pelvic inflammatory disease among young, single, sexually active women," *Sexually Transmitted Diseases*, vol. 33, no. 3, pp. 137–142, 2006.

[16] W. Hook, C. Spitters, C. Reichart, T. Neumann, and T. Quinn, "Use of cell culture and a rapid diagnostic assay for *Chlamydia trachomatis* screening," *The Journal of the American Medical Association*, vol. 272, no. 11, pp. 867–870, 1994.

[17] W. Geisler, C. Wang, S. Morrison, C. Black, C. Bandea, and W. Hook III., "The natural history of untreated *Chlamydia trachomatis* infection in the interval between screening and returning for treatment," *Sexually Transmitted Diseases*, vol. 35, no. 2, pp. 119–123, 2008.

[18] L. Bachmann, C. Richey, K. Waites, J. Schwebke, and W. Hook III., "Patterns of *Chlamydia trachomatis* testing and follow-up at a University Hospital Medical Center," *Sexually Transmitted Diseases*, vol. 26, no. 9, pp. 496–509, 1999.

[19] J. den Hartog, J. Land, F. Stassen, M. E. P. Slobbe-van Drunen, A. G. H. Kessels, and C. A. Bruggeman, "The role of chlamydia genus-specific and species-specific IgG antibody testing in predicting tubal disease in subfertile women," *Human Reproduction*, vol. 19, no. 6, pp. 1380–1384, 2004.

[20] S. Safrin, J. Schachter, D. Dahrouge, and R. Sweet, "Long-term sequelae of acute pelvic inflammatory disease. A retrospective cohort study," *American Journal of Obstetrics and Gynecology*, vol. 166, no. 4, pp. 1300–1305, 1992.

[21] R. Ness, D. Soper, H. Richter et al., "Chlamydia antibodies, chlamydia heat shock protein, and adverse sequelae after pelvic inflammatory disease: the PID Evaluation and Clinical Health (PEACH) Study," *Sexually Transmitted Diseases*, vol. 35, no. 2, pp. 129–135, 2008.

[22] C. Michel, C. Sonnex, C. Carne et al., "*Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies," *Journal of Clinical Microbiology*, vol. 45, no. 5, pp. 1395–1402, 2007.

[23] J. Paavonen, R. Aine, K. Teisala, P. Heinonen, and R. Punnonen, "Comparison of endometrial biopsy and peritoneal fluid cytologic testing with laparoscopy in the diagnosis of acute pelvic inflammatory disease," *American Journal of Obstetrics and Gynecology*, vol. 151, no. 5, pp. 645–650, 1985.

[24] B. Taylor, R. Ness, T. Darville, and C. Haggerty, "Microbial correlates of delayed care for pelvic inflammatory disease," *Sexually Transmitted Diseases*, vol. 38, no. 5, pp. 434–438, 2011.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 525182, 9 pages
doi:10.1155/2011/525182

Review Article

Antichlamydial Antibodies, Human Fertility, and Pregnancy Wastage

Amanda J. Stephens, Mira Aubuchon, and Danny J. Schust

Department of Obstetrics, Gynecology and Women's Health, University of Missouri School of Medicine, 500 North Keene Street, Suite 203, Columbia, MO 65201, USA

Correspondence should be addressed to Danny J. Schust, schustd@health.missouri.edu

Received 1 May 2011; Accepted 21 June 2011

Academic Editor: Thomas Cherpes

Genital infections with *Chlamydia trachomatis (C. trachomatis)* continue to be a worldwide epidemic. Immune response to chlamydia is important to both clearance of the disease and disease pathogenesis. Interindividual responses and current chlamydial control programs will have enormous effects on this disease and its control strategies. Humoral immune response to *C. trachomatis* occurs in humans and persistent antibody levels appear to be most directly correlated with more severe and longstanding disease and with reinfection. There is a close correlation between the presence of antichlamydial antibodies in females and tubal factor infertility; the closest associations have been found for antibodies against chlamydial heat shock proteins. The latter antibodies have also been shown to be useful among infertile patients with prior ectopic pregnancy, and their presence has been correlated with poor IVF outcomes, including early pregnancy loss. We review the existing literature on chlamydial antibody testing in infertile patients and present an algorithm for such testing in the infertile couple.

1. Introduction

Chlamydia trachomatis (C. trachomatis) infection is one of the most prevalent sexually transmitted diseases in the world. There were 409.2 cases per 100,000 population reported in the United States in 2009 [1]. *C. trachomatis* is a common cause of urethritis, epididymitis, prostatitis, cervicitis, pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility (TFI). As many as 80% of cases are asymptomatic, particularly among females. This leads to continued transmission of the infection to sexual partners and the opportunity for chronic infection.

Pelvic inflammatory disease (PID), an ascending infection from the cervix to the peritoneal cavity, is diagnosed in more than 800,000 women annually in the United States [2]. The most widely accepted microbial etiologies of PID are *Neisseria gonorrhoeae* and *C. trachomatis* [2–4]; still, other pathogens have been implicated and the final disease is almost certainly polymicrobial. While *C. trachomatis* infection may be a causative factor in up to 40% of cases of PID [5], fairly few women with *C. trachomatis* in the lower genital tract will progress to frank PID. The occurrence of symptomatic PID after untreated *C. trachomatis* infections may vary by population and time of followup but ranges between less than 2 and 9.5% [6, 7]. Most infected women will spontaneously clear their infections, although such clearance may take well over a year after infection [6, 8]. Women who do not clear their infections may suffer ascending infection and expansion into the full PID syndrome.

Once inflammation occurs in the fallopian tube, epithelial degeneration and deciliation of cells occur along the tube [2] (Figure 1 [9]). Edema in the fallopian tube exacerbates the intraluminal agglutination that occurs with endosalpingitis and leads to clubbing of the fimbriae and partial or complete tubal obstruction. Peritonitis caused by *C. trachomatis* can cause fibrinous exudates on the serosal surface of the uterus, fallopian tubes, and ovaries that fuses those structures to themselves and to surrounding bowel and omentum [2]. These adhesions are frequently associated with chronic pelvic pain. Each subsequent episode of PID doubles the risk for tubal factor infertility. Tubal pathology accounts for approximately 14% of subfertility [10]. Most women with

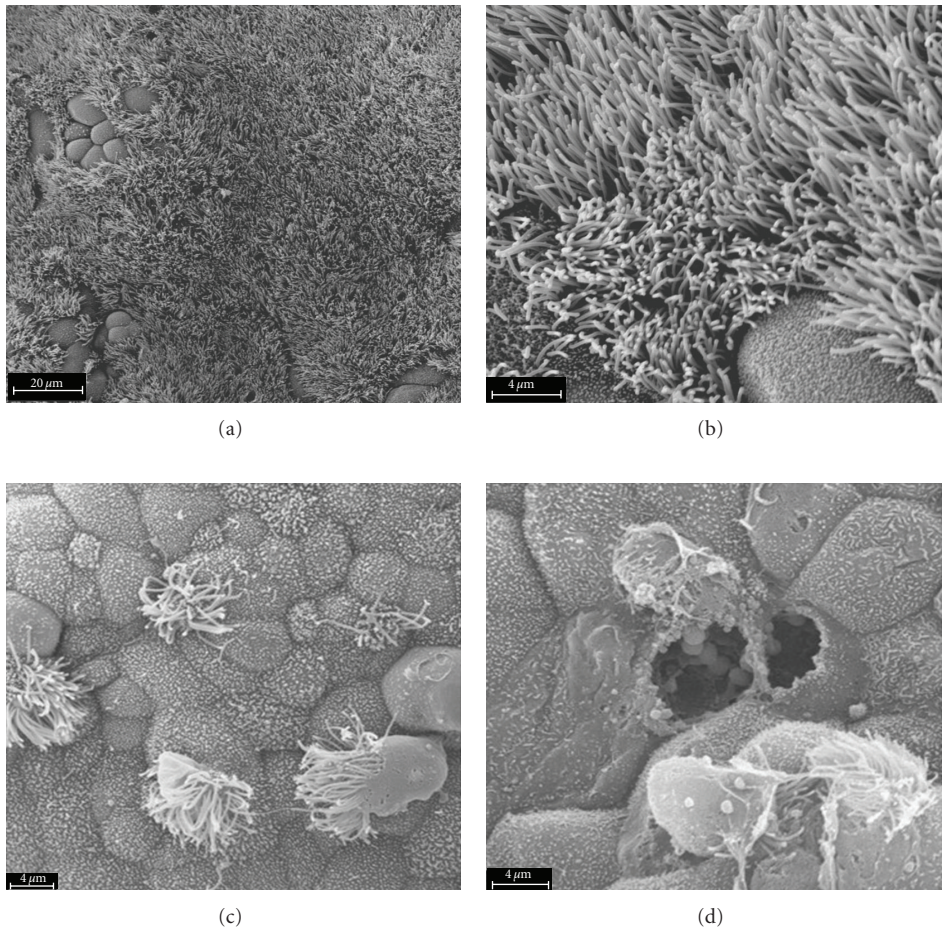


Figure 1: The effects of *C. trachomatis* infection on human fallopian tubal morphology. Human fallopian tubes in organ culture were left uninfected (a and b) or were infected with *C. trachomatis* serovar D (c and d). Ultrastructural analysis of the intratubal architecture uses scanning electron microscopy. Uninfected tubes are densely ciliated and contain intact secretory cells. The mucosal surface of *C. trachomatis*-infected tubes show remarkable deciliation and cellular disruption ([9], and reproduced with permission).

tubal occlusion have no known history of sexually transmitted infections. Evaluation of tubal infertility may include serologic studies, hysterosalpingography, and laparoscopy. Intrauterine dye infusion during laparoscopy is the gold standard for assessing tubal occlusion, endometriosis, or pelvic adhesions in infertility patients. Laparoscopy, however, is a costly invasive test that has risk for complications. Hysterosalpingogram (HSG), a less costly and less complicated imaging modality, has a sensitivity of 65–96% and specificity of 73–83% for detecting tubal pathology [10–12]. This paper aims to evaluate the serologic tests available for *C. trachomatis* and their associations with TFI.

2. Pathogenesis of Disease

C. trachomatis is an obligate intracellular bacterium that produces a wide variety of clinical pathologies. Serovars D through K are pathologic to mucosal epithelial cells of the urogenital tract [13]. Erythema, edema, and mucopurulent discharge can be seen on physical exam during acute infection [14]. Urethritis, epididymitis, prostatitis, cervicitis, and pelvic inflammatory disease can develop following infection. With chronic infection, cellular changes including fibrosis and mononuclear cell infiltration lead to increased risk for ectopic pregnancy and TFI [14]. Both persistent infection and re-infection with *C. trachomatis* may be associated with worsening long-term sequelae, although the former appears to be the most consequential [15]. The ability of *C. trachomatis* to transform repeatedly from the resting form (elementary body; EB) to the replicative form (reticulate body; RB) enhances survival of the organism in the reproductive tract [16] (Figure 2 [17]). The EB of *C. trachomatis* attaches to the epithelial cell surface and incorporates into phagosomes that migrate to the distal region of the Golgi complex [13]. Lysosome fusion is prevented, and chlamydial infection averts immediate destruction. The EB then differentiates into the noninfectious but replicative reticulate body (RB) which further divides by binary fission [13]. Although *C. trachomatis* can partially evade immune detection [18–24] making infections fairly asymptomatic in many women, the infectious particles can be recognized by the host, with subsequent activation of host interferon (IFN-) γ and proinflammatory

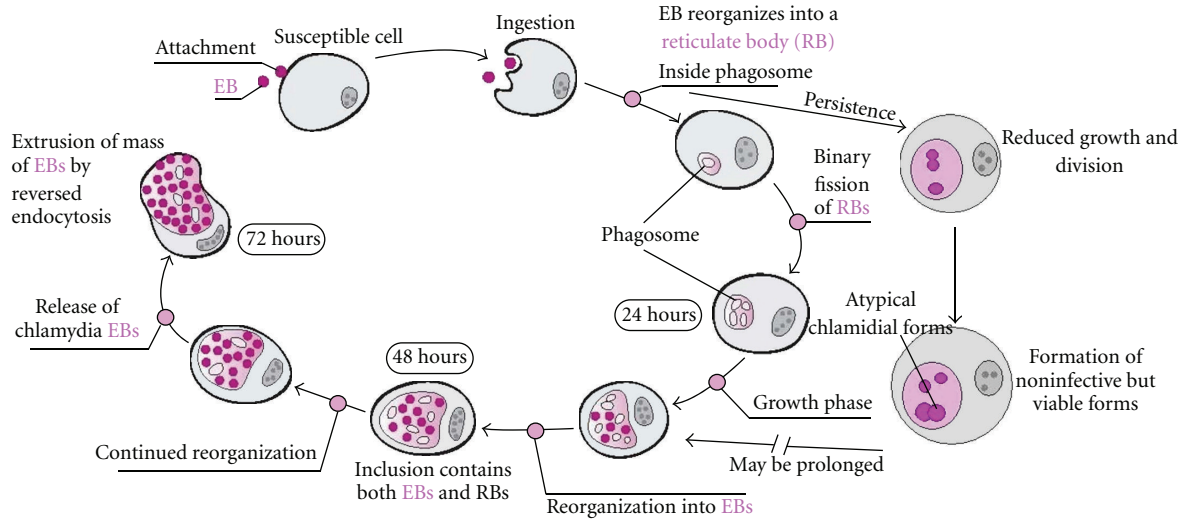


Figure 2: The life cycle of genital serovars of *C. trachomatis*. The chlamydial growth cycle involves transformation between distinct forms: the elementary body (EB) and the reticulate body (RB). The highly infectious EB attaches to nonciliated columnar or cuboidal epithelial cells and induces ingestion by the host cell. EB are metabolically inactive and represent the extracellular *C. trachomatis* growth form. Once ingested into a phagosome, fusion of the phagosome with the host lysosome is prevented, a highly unusual occurrence that ensures EB survival. The EB reorganizes within the phagosome into a metabolically active RB. RBs are noninfectious but can replicate and do so by binary fission. Several stimuli, including antibiotic and IFNγ exposure, can drive chlamydia into a persistent state, which lasts in vitro until removal of the exogenous stressor. If persistence is avoided, or if infection is reactivated from persistence, the RB will ultimately reorganize back into EB, which will be released from the host cell to infect surrounding epithelial cells (reproduced with permission [17]).

cytokine secretion [25, 26]. In response to interferon exposure in vitro, RB can enter a persistent and noninflammatory state [26, 27]. Persistence can also be driven by nonsterilizing antibiotic exposure in vitro [14, 25–27]. Although there remains no direct in vivo evidence for persistence in humans, clinical scenarios suggest that persistent infection may remain undetected for many years and reactivation may occur much later in life. Reactivation may sometimes occur in women with prior infections who are now no longer sexually active or in women who have had their fallopian tubes electively obstructed and no longer have a patent route from lower to upper genital tract [14, 25, 28]. In the persistent state, chlamydial heat shock protein 60 (CHSP60) genes are upregulated and released [13, 29, 30]. In humans, elevated antibody responses to CHSP60 have been strongly associated with PID, ectopic pregnancy, scarring trachoma, and tubal infertility [13, 25, 29, 30]. Long-term exposure to CHSP60 may lead to a loss of tolerance to cross-reactive endogenous human antigens and generation of immune responses to conserved amino acid sequences that are also expressed in homologous human hsp60 [13, 25, 29, 30]. This immunity can lead to immune responses against human hsp60 in the early embryo and potentially link *C. trachomatis* infections to spontaneous pregnancy loss (see the following).

It remains unclear why some women clear their infections, while others endure long-lasting, ascending, and possibly persistent infections. The human cytokine IFN-γ may play a central role in this enigma. IFN-γ secretion by infected cells and by those cells brought in to control infection is central to infection clearance [6, 31]. IFN-γ is also involved in the tissue damage associated with *C. trachomatis* infections [6, 31]. Finally, in vitro models use low to moderate level IFN-γ exposure to drive *C. trachomatis* persistence with reactivation of the developmental cycle occurring after removal of the exogenous IFN [27]. The level of immune response to *C. trachomatis* may be affected by many factors. A large initial inoculation of infectious organisms may tip the balance toward exuberant responses that may clear the infection but be associated with more extensive damage. Genetic differences among infected subjects may alter response to infection. Several investigators have now reported on the effects of single nucleotide polymorphisms (SNPs) in inflammatory mediators, including cytokines, on the susceptibility to *C. trachomatis* infection and on the severity of tubal damage incurred during such infections [32, 33]. The level of oxygen within the fallopian tubes of *C. trachomatis* infected women may alter the balance of IFN-mediated clearance versus damage [34]. Reinfection may drive adverse infection sequelae among women who have already developed amnestic responses to *C. trachomatis*. Although some women spontaneously lose antibody responses to chlamydial antigens [35], antichlamydial IgG antibodies frequently persist for prolonged periods of time, even among women who have been treated with antibiotics [36, 37].

2.1. C. trachomatis Antibodies and TFI. Several immunologic techniques have been employed to study the relationship between the results of *C. trachomatis* serologic testing and the severity of *C. trachomatis*-associated pathologies, including TFI (summarized in Table 1). The most commonly studied antibodies include those directed against chlamydial IgG and

Table 1: Role of antichlamydial antibody testing in male and female fertility.

	Method	Sens. (%)	Specif. (%)	PPV (%)	NPV (%)	Utility in females	Utility in males
CT IgG [22]	ELISA	72.7	77.7	—	—	Presence indicates previous or persistent *C. trachomatis* infection; associated with tubal damage; increased titers associated with more severe tubal damage; sens./specif. may be increased with the addition of HSG or laparoscopy	—
CT IgG [33]	ELISA	43.2	86.5	63.3	73.8		—
CT IgG [6]	MIF	74	93	94.8	69.8		—
CT IgG [5]	EIA	45	83	—	—		—
CT IgG [23] Titer > 1 : 256	WIF	69	85	78	78		—
CT HSP60 [33]	ELISA	59.1	77.9	59.1	77.9	Reflects chronic *C. trachomatis* infection; predicts TFI	—
CT HSP60 [7]	GST ELISA	56	95	—	—	Higher titers related to increased severity of tubal damage	—
+ClpP Ab [7]	GST ELISA	69	—	92	79	Improve sens./specif. in Ab based diagnosis of TFI	—
CT IgA [28, 29]	MIF/EIA	—	—	—	—	—	Reduces chances of achieving pregnancy; reduced motility of spermatozoa, increased number of dead spermatozoa
+CT IgG [28, 29]	MIF/EIA	—	—	—	—	—	Further reduce pregnancy rates, decrease sperm concentration, decrease number of progressive spermatozoa
CT HSP60 [29]	ELISA	—	—	—	—	—	Reduce spermatozoa motility

ELISA: enzyme-linked immunosorbent assay, EIA: enzyme immunoassay, WIF: whole-cell inclusion immunofluorescence assay, GST ELISA: glutathione *S*-transferase ELISA, MIF: microimmunofluorescence, Ab: antibody; Sens.: sensitivity, Specif.: specificity, PPV: positive predictive value, NPV: negative predictive value; TFI: tubal factor infertility; HSG: hysterosalpingogram.

CHSP60. The results of each of these investigations must be interpreted with caution, as the methodologies used to detect antibodies vary in their utility and the populations studied may vary in their genetic predisposition to immune responsivity and antibody production and persistence. Still the results appear to trend similarly. Elevated antichlamydial antibody levels can be found in >70% of women with tubal occlusion [12]. Malik et al. [38] evaluated IgG antibodies to *C. trachomatis* using enzyme-linked immunosorbent assays (ELISAs) and found that 63.6% of those with positive IgG serologies had tubal occlusion on HSG. This results in a sensitivity of 72.7% and specificity of 44.4% for tubal occlusion [38]. Perquin et al. [10] evaluated the presence of antichlamydial IgG antibodies using species-specific enzyme immunoassay (EIA) and compared these levels to findings of HSG and at laparoscopy. The sensitivity and specificity for IgG antibodies using EIA in predicting tubal pathology were found to be 45% and 83%, respectively [10]. Akande et al. [39] evaluated antichlamydial IgG antibodies using single-antigen inclusion tests and indirect immunofluorescence (whole-cell inclusion immunofluorescence; WIF). Antibody titers were found to be significantly higher among infertile women who had previously conceived compared to those with primary infertility [39]. This seemingly contradictory finding may be related to increased risk factors for sexually transmitted infections, including increased numbers of sexual partners, in those with secondary infertility, or with higher prevalence of other causes of infertility (e.g., anovulation or endometriosis) in those with primary infertility. Titers were significantly higher among those with a history of PID and those with documented tubal pathology. A linear relationship was observed between antibody titer level and the likelihood of tubal damage. Patients with the highest titers (>1 : 4096) had a 100% rate of tubal damage, and 73.1% of those had severe tubal damage [39]. Negative antibody testing did not preclude the diagnosis of tubal damage. den Hartog et al. [40] evaluated subfertile women with chlamydial antibody testing (CAT) involving antichlamydial Ig ELISA and high-sensitivity C-reactive protein (hsCRP) ELISA and compared the results to tubal evaluations using HSG. Seropositivity for IgG antibodies reflects previous *C. trachomatis* infection, while the presence of hsCRP reflects

persistence of the infection. They found that the addition of HSG was of limited value in predicting tubal pathology in women who were CAT positive or CAT and hsCRP positive.

Rodgers et al. [12] reported that TFI patients had higher titer levels of antichlamydial antibodies than either women in infertile control couples without TFI or in fertile control couples [12]. They also found that antibodies against ClpP were significantly higher in the TFI group compared to controls. ClpP is a proteolytic subunit of the ATP-dependent Clp protease complex that is part of a highly conserved serine protease family in eukaryotes and bacteria [12]. Chlamydial ClpP displays a 45% amino acid sequence identity with its homolog in humans. It is hypothesized that human antichlamydial ClpP antibodies can recognize cross-reactive epitopes and attack human ClpP in tissues [12].

Heat shock proteins (HSPs) are stress response proteins found in humans, animals, and bacteria. Expression of HSPs increases with temperature changes, ischemia, or hypoxia [12, 29, 30]. They have also been implicated in cell transformation and the development of metastatic potential and multidrug resistance [41]. HSPs contain amino acid regions that are highly conserved among the organisms that express them. Antibodies to CHSP60 have been linked to TFI in numerous studies [12, 25, 29, 30, 41]. CHSP60 stimulates inflammatory responses, including activation of macrophages and epithelial cells to secrete the inflammatory cytokines, IFN-γ and tumor necrosis factor-α (TNF-α), as well as other proinflammatory mediators [12, 41]. Stimulation of endothelial cells, smooth muscle cells, and macrophages leads to the production of adhesion factors and proinflammatory cytokines by activation of nuclear factor-κB (NF-κB) [41]. Jakus et al. [42] reported on the presence of anti-CHSP60 IgG antibodies in the follicular fluid of patients who had previously undergone IVF. Anti-CHSP60 antibodies were detected in 74.1% of women with implantation failure; 47.9% of those with 1–3 implantations per IVF cycle were anti-CHSP60 positive. Among women with documented tubal occlusion, 69.5% were antibody positive, while only 49.7% of women with other causes of infertility had anti-CHSP60 antibodies [42]. In another study, the presence of anti-HSP60 antibodies predicted TFI with a sensitivity of 56% but a specificity of 95%. Inclusion of anti-ClpP antibody testing increased the sensitivity to 69% [12]. The negative predictive value of ClpP and HSP60 for TFI was 79% and positive predictive value 92% [43]. Keltz et al. [11] assessed the sensitivity and specificity of HSG alone and HSG combined with CAT to detect tubal occlusion when compared to laparoscopy. HSG alone had sensitivity of 78% and specificity of 82%, while CAT alone had excellent positive predictive value of 94.8% but a poor negative predictive value of 69.8% due to its low sensitivity of 74%. This sensitivity increased to 97.3% when CAT results were combined with those of HSG. Laparoscopy, however, found pelvic pathologies other than tubal occlusion in 33–68% of patients with a normal HSG [11].

El Hakim et al. [44] evaluated 408 women with documented tubal damage on laparoscopy and compared the severity of damage with antibody titer levels using WIF. Similar to the results of the Keltz study [11], the severity of tubal damage was found to correlate significantly with increasing serum antibody levels [11, 44]. Bipolar and distal tubal occlusion were found to have the highest antibody titers [44].

Van Tetering et al. [45] evaluated 711 women with known anovulation and compared their antichlamydial antibody titers using ELISA with tubal pathology diagnosed using either HSG or laparoscopy. CAT screening yielded a sensitivity and specificity for tubal damage of 20% and 89%, respectively. However, the prevalence of CAT positivity was <5%, suggesting limited value for CAT screening in infertile women with known etiologic factors, particularly anovulation [45].

2.2. C. trachomatis Antibodies and Male Factor Infertility. While women can develop PID after *C. trachomatis* infections, men can develop urethritis, epididymitis, orchitis, and proctitis. Epididymitis can lead to canalicular system damage and obstructive azoospermia, although such severe post-chlamydial outcomes are uncommon. *C. trachomatis* infections more commonly result in the generation of antisperm antibodies and changes in semen quality that diminish rather than prevent male fertility. Detection of anti-*C. trachomatis* IgA and IgG antibodies, but not anti-HSP60 IgG antibodies, in male serum has been associated with poor semen characteristics and pregnancy rates regardless of female partner antibody status [46, 47] (summarized in Table 1). In one investigation, the presence of serum antichlamydial IgA, alone or in combination with IgG, correlates with reduced concentration and progressive motility of spermatozoa, an increase in the number of dead spermatozoa, poor sperm morphology, and a higher prevalence of leukocytospermia [46]. Men with IgG alone or IgA alone decrease their chance of achieving pregnancy by a third; men with both serum antibodies decrease their chances by almost two-thirds [46]. Interestingly, these reductions were completely surmounted through the use of in vitro fertilization (IVF). Not all investigators have replicated these findings. Eggert-Kruse et al. [48] examined male serum and seminal plasma from subfertile couples for antichlamydial IgA, IgG, and IgM antibodies using chlamydial lipopolysaccharide- (LPS-) directed ELISA. Although the presence of IgA antibodies in seminal fluid was associated with antibody detection in the serum of the female partners, the findings did not correlate with reduced sperm count or motility or subsequent fertilizing capacity [48].

2.3. C. trachomatis Antibodies and Pregnancy Outcomes. Antibodies to *C. trachomatis* and CHSP60 are strongly associated with TFI. Individuals with known TFI commonly undergo in vitro fertilization to overcome their tubal pathology, but they may still be at risk for adverse obstetric outcomes such as spontaneous abortion or biochemical pregnancy [13]. Among 174 women with normal fallopian tubes at laparoscopy who were followed for 3 years, the presence of antichlamydial antibodies using immunoflourescence (38.5%) was not predictive of obstetric outcomes [49]. The risk appears to be higher in the presence of antibodies specific to CHSP60. Human hsp60 is expressed during early embryo development and normally does not trigger an immune

response [13, 42]. Heat shock proteins of various species contain a highly conserved region of amino acids. Sensitization to this conserved region in CHSP60 can result in reactivation of previously tolerized HSP60-specific lymphocytes. This may, in turn, compromise fetal or maternal cell viability via the direct activity of anti-HSP60 antibodies and/or the accompanying proinflammatory response. In 1999, Witkin [50] reported that women with cervical antichlamydial and anti-CHSP60 IgA antibodies were less likely to have a live birth after in vitro fertilization than their counterparts who did not have these antibodies. Patients undergoing IVF do not require patent or normal fallopian tubes. The rate of very early pregnancy loss was 3 times greater among those women who were anti-CHSP60 positive. Further, incubation of embryos in media containing human sera positive for anti-human HSP60 antibodies inhibited embryo development [50]. Supporting these results, Jakus et al. [42] studied 253 IVF patients and demonstrated lower implantation rates among women with follicular fluid anti-CHSP60 antibodies when compared with antibody negative controls. No differences were detected in the number of oocytes collected or the percentage that fertilized.

Among women less than 35 years of age with a history of ectopic pregnancy treated with salpingectomy, the presence of serum anti-CHSP60 antibodies predicted lower spontaneous conception rates and decreased term delivery rates [51]. The same study reported that circulating IgG antibodies to a conserved epitope of CHSP60 (amino acids 260–271) were associated with decreased spontaneous fertility, repeated ectopic pregnancy, and adverse subsequent pregnancy outcome, suggesting that damage to the remaining tube may have occurred prior to the salpingectomy [51]. Women without antibodies to CHSP60 260–271 were five times more likely to have documented intrauterine conceptions and term deliveries when compared to those with positive serologies [51]. Women with positive serologies might therefore consider IVF after a first ectopic pregnancy to improve conception and pregnancy outcomes.

2.4. Use of Chlamydial Antibody Screening in Couples with Infertility. Despite several continuing controversies, the existing data on the relationship between antichlamydial antibodies and tubal factor infertility make consideration of algorithms for screening possible (Figure 3). Chlamydial antibody screening is inexpensive when compared to other methods of tubal evaluation (HSG) but offers similar or improved sensitivity and specificity. HSG is more likely to be associated with adverse sequelae among women with chlamydial antibodies [40].

In our proposed algorithm, couples presenting with infertility would be screened first with careful histories and physical exams. If the female partner has a history of ectopic pregnancy, testing for anti-CHSP60 antibodies could be performed. Those with positive antibodies may be counseled to consider progression towards IVF to optimize their chance for a live birth. Couples without such a history would undergo documentation of ovulatory function and semen analysis. If the female was anovulatory, but the semen analysis was normal, the woman could consider ovulation induction and timed intercourse for 3-4 cycles prior to further interventions. If the woman was anovulatory or had normal ovulatory function, but the semen analysis revealed severe abnormalities, IVF would be recommended. If the semen analysis revealed persistent mild to moderate abnormalities, referral to an urologist could be considered and 3-4 cycles of timed intrauterine insemination undertaken prior to further evaluation. This would be combined with ovulation induction in anovulatory women. Couples with normal ovulation and normal semen parameters would be offered antichlamydial antibody testing (CAT). The significant body of literature linking CAT to TFI using anti- chlamydial major outer membrane proteins (MOMPs) antibodies warrants preliminary screening with standard CAT. Despite reports that CAT antibody levels can be associated with severity of tubal disease, the wide variations in antibody levels among affected patients reduce our enthusiasm for using antibody titer levels in management decisions at this time [44]. If CAT using MOMP is positive, laparoscopy would be recommended; if negative, testing for antibodies against CHSP60 (and possibly chlamydial Clp protease; ClpP) would be performed. Those positive for antichlamydial antibodies would proceed directly to laparoscopy for diagnosis and treatment. If normal fallopian tubes were found at laparoscopy, the couple could be treated using standard protocols for unexplained infertility [49]. Those negative for antibodies could also be treated for unexplained infertility.

This suggested algorithm may be improved as additional antibody targets on chlamydia are identified and may need to be modified in light of current worldwide efforts to control the chlamydia epidemic [6, 52, 53]. Accompanying these control efforts are decreases in postinfection sequelae but continued increases in infection prevalence. It is possible that early identification and treatment of *C. trachomatis* infections is interrupting not only the destructive immune response to the pathogen but the protective response as well, a phenomenon that has been called "the arrested immunity hypothesis" [54]. Partner treatment may therefore be an important part of control paradigms as reinfection may become increasingly common. Does this arrested immunity result in reduced levels of protective IFN-γ that may promote persistence in some individuals? Arrested immunity might hinder antibody responses and make persistence of antibodies less common among previously infected but treated women. This may have little effect on screening programs if these women also avoid persistent infections. We do not presently have a definitive answer to these questions. Antibiotics such as penicillin and sulfonamides have been demonstrated to drive the development of chlamydial persistence in vitro [55]. Incomplete antibiotic therapy in areas that do not have availability of single-dose regimens might be predicted to promote persistence in vivo. Continued surveillance of chlamydial antibody screening programs for infertile couples will be key to monitoring the effects of control programs on the utility of screening.

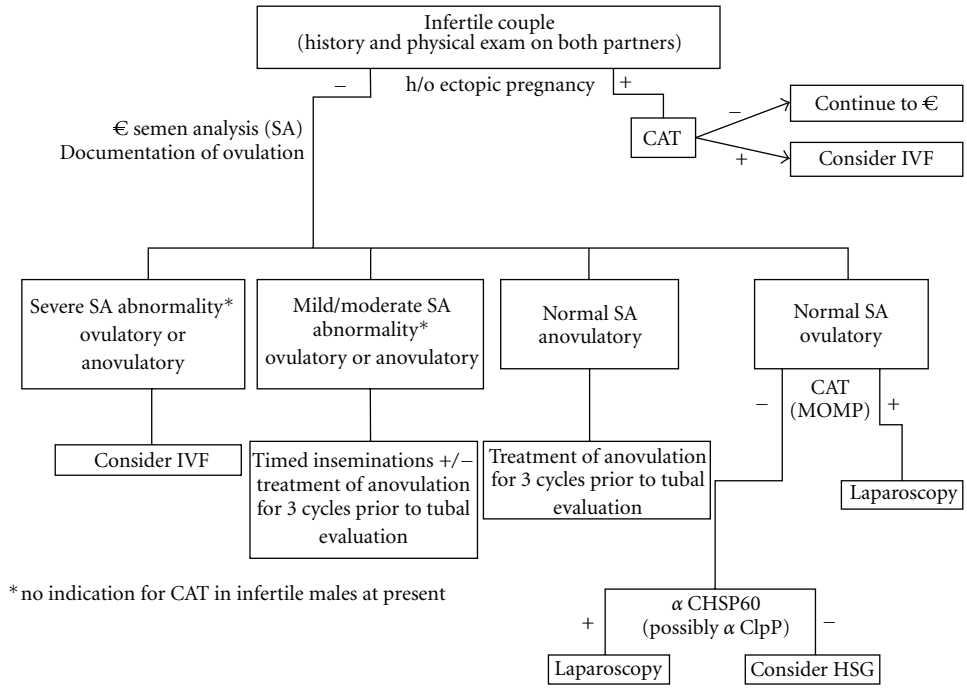


Figure 3: A proposed algorithm for use of chlamydial antibody screening in infertile couples.

3. Conclusion

C. trachomatis is one of the most prevalent sexually transmitted infections in the United States and worldwide. Long-term sequelae of *C. trachomatis* infection include pelvic inflammatory disease, tubal factor infertility, and risk of ectopic pregnancy. Antibody testing for both antichlamydial IgG and anti-CHSP60 has been found to be associated with TFI. Increasingly high titers of antichlamydial IgG and anti-CHSP60 antibodies have been correlated with increasing severity of tubal damage when evaluated using HSG or laparoscopy. While sensitivity and specificity for CAT are comparable to that of HSG alone, CAT is less cost prohibitive and has less risks than either HSG or laparoscopy. CAT may be a valuable screening test prior to laparoscopy in infertility patients. Chlamydial antibodies have also been associated with male factor infertility, including reductions in sperm motility and total sperm counts. Chlamydial HSP60 antibodies have also been shown cross-react with human HSP60. This may lead to immune destruction of the early embryo and pregnancy wastage among women previously infected with *C. trachomatis*. Chlamydia antibody testing is a low-risk screening modality with sensitivity and specificity comparable to HSG and should be considered in the initial infertility evaluation. Limitations of CAT include (1) an inability to identify women with noninfectious causes of TFI, such as endometriosis, previous pelvic surgeries, or peritonitis, requiring these women to proceed with HSG or laparoscopy; (2) the possibility of declining antibody titers over time; (3) the ability to detect high titers among some women with normal appearing fallopian tubes; (4) the probability that additional antibody targets will be defined that improve screening sensitivity and specificity; (5) the unknown effects of chlamydial control programs on the utility of antibody testing among infertile couples.

References

[1] CDC, "Sexually-transmitted diseases: Chlamydia," 2010, http://www.cdc.gov/std/chlamydia.

[2] D. E. Soper, "Pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 116, no. 2, part 1, pp. 419–428, 2010.

[3] D. E. Soper, N. J. Brockwell, and H. P. Dalton, "Microbial etiology of urban emergency department acute salpingitis: treatment with ofloxacin," *American Journal of Obstetrics and Gynecology*, vol. 167, no. 3, pp. 653–660, 1992.

[4] R. L. Sweet, D. L. Draper, and J. Schachter, "Microbiology and pathogenesis of acute salpingitis as determined by laparoscopy: what is the appropriate site to sample?" *American Journal of Obstetrics and Gynecology*, vol. 138, no. 7, part 2, pp. 985–989, 1980.

[5] U.S. Department of Health and Human Services, "Chlamydia," 2010, http://www.womenshealth.gov/faq/chlamydia.pdf. .

[6] S. L. Gottlieb, D. H. Martin, F. Xu, G. I. Byrne, and R. C. Brunham, "Summary: the natural history and immunobiology of *Chlamydia trachomatis* genital infection and implications for chlamydia control," *Journal of Infectious Diseases*, vol. 201, supplement 2, pp. S190–S204, 2010.

[7] P. Oakeshott, S. Kerry, A. Aghaizu et al., "Randomised controlled trial of screening for *Chlamydia trachomatis* to prevent pelvic inflammatory disease: the POPI (prevention of pelvic infection) trial," *BMJ*, vol. 340, Article ID c1642, 2010.

[8] W. M. Geisler, "Duration of untreated, uncomplicated *Chlamydia trachomatis* genital infection and factors associated with chlamydia resolution: a review of human studies," *Journal*

of Infectious Diseases, vol. 201, supplement 2, pp. S104–S113, 2010.

[9] A. Baczynska, P. Funch, J. Fedder, H. J. Knudsen, S. Birkelund, and G. Christiansen, "Morphology of human Fallopian tubes after infection with Mycoplasma genitalium and Mycoplasma hominis—in vitro organ culture study," *Human Reproduction*, vol. 22, no. 4, pp. 968–979, 2007.

[10] D. A. Perquin, M. F. C. Beersma, A. J. M. de Craen, and F. M. Helmerhorst, "The value of *Chlamydia trachomatis*-specific IgG antibody testing and hysterosalpingography for predicting tubal pathology and occurrence of pregnancy," *Fertility and Sterility*, vol. 88, no. 1, pp. 224–226, 2007.

[11] M. D. Keltz, P. S. Gera, and M. Moustakis, "Chlamydia serology screening in infertility patients," *Fertility and Sterility*, vol. 85, no. 3, pp. 752–754, 2006.

[12] A. K. Rodgers, J. Wang, Y. Zhang et al., "Association of tubal factor infertility with elevated antibodies to *Chlamydia trachomatis* caseinolytic protease P," *American Journal of Obstetrics and Gynecology*, vol. 203, no. 5, pp. 494.e7–494.e14, 2010.

[13] I. M. Linhares and S. S. Witkin, "Immunopathogenic consequences of *Chlamydia trachomatis* 60 kDa heat shock protein expression in the female reproductive tract," *Cell Stress and Chaperones*, vol. 15, no. 5, pp. 467–473, 2010.

[14] R. C. Brunham and M. L. Rekart, "Considerations on *Chlamydia trachomatis* disease expression," *FEMS Immunology and Medical Microbiology*, vol. 55, no. 2, pp. 162–166, 2009.

[15] J. E. den Hartog, J. A. Land, F. R. M. Stassen, A. G. H. Kessels, and C. A. Bruggeman, "Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility," *Human Reproduction*, vol. 20, no. 4, pp. 986–990, 2005.

[16] J. Paavonen and M. Lehtinen, "Chlamydial pelvic inflammatory disease," *Human Reproduction Update*, vol. 2, no. 6, pp. 519–529, 1996.

[17] L. J. Heffner and D. J. Schust, "Sexually transmitted diseases of bacterial origin," in *The Reproductive System at a Glance*, Wiley-Blackwell, Oxford, UK, 2010.

[18] K. Kawana, J. Matsumoto, S. Miura et al., "Expression of CD1d and ligand-induced cytokine production are tissue specific in mucosal epithelia of the human lower reproductive tract," *Infection and Immunity*, vol. 76, no. 7, pp. 3011–3018, 2008.

[19] K. Kawana, A. J. Quayle, M. Ficarra et al., "CD1d degradation in *Chlamydia trachomatis*-infected epithelial cells is the result of both cellular and chlamydial proteasomal activity," *Journal of Biological Chemistry*, vol. 282, no. 10, pp. 7368–7375, 2007.

[20] G. Le Negrate et al., "ChlaDub1 of Chlamydia trachomatis suppresses NF-kappaB activation and inhibits IkappaBalpha ubiquitination and degradation," *Cellular Microbiology*, vol. 10, no. 9, pp. 1879–1892, 2008.

[21] B. Mei, K. Du, Z. Huo, Y. Zou, and P. Yu, "Discrepant effects of *Chlamydia trachomatis* infection on MICA expression of HeLa and U373 cells," *Infection, Genetics and Evolution*, vol. 10, no. 6, pp. 740–745, 2010.

[22] D. E. Nelson, D. P. Virok, H. Wood et al., "Chlamydial IFN-γ immune evasion is linked to host infection tropism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 30, pp. 10658–10663, 2005.

[23] G. Zhong, T. Fan, and L. Liu, "Chlamydia inhibits interferon γ-inducible major histocompatibility complex class II expression by degradation of upstream stimulatory factor 1," *Journal of Experimental Medicine*, vol. 189, no. 12, pp. 1931–1938, 1999.

[24] G. Zhong, L. Liu, T. Fan, P. Fan, and H. Ji, "Degradation of transcription factor RFX5 during the inhibition of both constitutive and interferon γ-inducible major histocompatibility complex class I expression in chlamydia-infected cells," *Journal of Experimental Medicine*, vol. 191, no. 9, pp. 1525–1534, 2000.

[25] R. J. Hogan, S. A. Mathews, S. Mukhopadhyay, J. T. Summersgill, and P. Timms, "Chlamydial persistence: beyond the biphasic paradigm," *Infection and Immunity*, vol. 72, no. 4, pp. 1843–1855, 2004.

[26] P. B. Wyrick, "*Chlamydia trachomatis* persistence in vitro: an overview," *Journal of Infectious Diseases*, vol. 201, supplement 2, pp. S88–S95, 2010.

[27] P. Mpiga and M. Ravaoarinoro, "*Chlamydia trachomatis* persistence: an update," *Microbiological Research*, vol. 161, no. 1, pp. 9–19, 2006.

[28] M. Levgur and R. Duvivier, "Pelvic inflammatory disease after tubal sterilization: a review," *Obstetrical and Gynecological Survey*, vol. 55, no. 1, pp. 41–50, 2000.

[29] A. Hjelholt, G. Christiansen, T. G. Johannesson, H. J. Ingerslev, and S. Birkelund, "Tubal factor infertility is associated with antibodies against *Chlamydia trachomatis* heat shock protein 60 (HSP60) but not human HSP60," *Human Reproduction*, vol. 26, no. 8, pp. 2069–2076, 2011.

[30] A. Kinnunen, J. Paavonen, and H. M. Surcel, "Heat shock protein 60 specific T-cell response in chlamydial infections," *Scandinavian Journal of Immunology*, vol. 54, no. 1-2, pp. 76–81, 2001.

[31] T. Darville and T. J. Hiltke, "Pathogenesis of genital tract disease due to *Chlamydia trachomatis*," *Journal of Infectious Diseases*, vol. 201, no. 2, pp. S114–S125, 2010.

[32] H. Öhman, A. Tiitinen, M. Halttunen, M. Lehtinen, J. Paavonen, and H. M. Surcel, "Cytokine polymorphisms and severity of tubal damage in women with chlamydia-associated infertility," *Journal of Infectious Diseases*, vol. 199, no. 9, pp. 1353–1359, 2009.

[33] W. Wang, F. R. Stassen, H. M. Surcel et al., "Analyses of polymorphisms in the inflammasome-associated NLRP3 and miRNA-146A genes in the susceptibility to and tubal pathology of *Chlamydia trachomatis* infection," *Drugs of Today*, vol. 45, supplement B, pp. 95–103, 2009.

[34] A. Roth, P. König, V. G. Zandbergen et al., "Hypoxia abrogates antichlamydial properties of IFN-γ in human fallopian tube cells in vitro and ex vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 45, pp. 19502–19507, 2010.

[35] A. P. Gijsen, J. A. Land, V. J. Goossens, M. E. P. Slobbe, and C. A. Bruggeman, "Chlamydia antibody testing in screening for tubal factor subfertility: the significance of IgG antibody decline over time," *Human Reproduction*, vol. 17, no. 3, pp. 699–703, 2002.

[36] J. Henry-Suchet, M. Askienazy-Elbhar, M. Thibon, C. Revol, and B. A. Akue, "Post-therapeutic evolution of serum chlamydial antibody titers in women with acute salpingitis and tubal infertility," *Fertility and Sterility*, vol. 62, no. 2, pp. 296–304, 1994.

[37] J. Paavonen, "*Chlamydia trachomatis* infections of the female genital tract: state of the art," *Annals of Medicine*. In press.

[38] A. Malik, S. Jain, M. Rizvi, I. Shukla, and S. Hakim, "*Chlamydia trachomatis* infection in women with secondary infertility," *Fertility and Sterility*, vol. 91, no. 1, pp. 91–95, 2009.

[39] V. A. Akande, L. P. Hunt, D. J. Cahill, E. O. Caul, W. C. L. Ford, and J. M. Jenkins, "Tubal damage in infertile women: prediction using chlamydia serology," *Human Reproduction*, vol. 18, no. 9, pp. 1841–1847, 2003.

[40] J. E. den Hartog, C. M. J. G. Lardenoije, J. L. Severens, J. A. Land, J. L. H. Evers, and A. G. H. Kessels, "Screening strategies

for tubal factor subfertility," *Human Reproduction*, vol. 23, no. 8, pp. 1840–1848, 2008.

[41] C. Campanella, A. Marino Gammazza, L. Mularoni, F. Cappello, G. Zummo, and V. Di Felice, "A comparative analysis of the products of GROEL-1 gene from *Chlamydia trachomatis* serovar D and the HSP60 var1 transcript from Homo sapiens suggests a possible autoimmune response," *International Journal of Immunogenetics*, vol. 36, no. 1, pp. 73–78, 2009.

[42] S. Jakus, A. Neuer, S. Dieterle, A. M. Bongiovanni, and S. S. Witkin, "Antibody to the *Chlamydia trachomatis* 60kDa heat shock protein in follicular fluid and in vitro fertilization outcome," *American Journal of Reproductive Immunology*, vol. 59, no. 2, pp. 85–89, 2008.

[43] A. K. Rodgers, J. Wang, Y. Zhang et al., "Association of tubal factor infertility with elevated antibodies to *Chlamydia trachomatis* caseinolytic protease P," *American Journal of Obstetrics and Gynecology*, vol. 203, no. 5, pp. 494.e7–494.e14, 2010.

[44] E. A. El Hakim, U. D. Gordon, and V. A. Akande, "The relationship between serum Chlamydia antibody levels and severity of disease in infertile women with tubal damage," *Archives of Gynecology and Obstetrics*, vol. 281, no. 4, pp. 727–733, 2010.

[45] E. A. A. van Tetering, P. Bourdrez, C. A. M. Koks et al., "Routine chlamydia antibody testing is of limited use in subfertile women with anovulation," *Reproductive BioMedicine Online*, vol. 14, no. 3, pp. 322–327, 2007.

[46] A. Idahl, L. Abramsson, U. Kumlin, J. A. Liljeqvist, and J. I. Olofsson, "Male serum *Chlamydia trachomatis* IgA and IgG, but not heat shock protein 60 IgG, correlates with negatively affected semen characteristics and lower pregnancy rates in the infertile couple," *International Journal of Andrology*, vol. 30, no. 2, pp. 99–107, 2007.

[47] P. Joki-Korpela, N. Sahrakorpi, M. Halttunen, H. M. Surcel, J. Paavonen, and A. Tiitinen, "The role of *Chlamydia trachomatis* infection in male infertility," *Fertility and Sterility*, vol. 91, supplement 4, pp. 1448–1450, 2009.

[48] W. Eggert-Kruse, M. Weltin, and T. Strowitzki, "Are Chlamydial lipopolysaccachide-directed antibodies in seminal plasma or serum clinically significant during investigation of male infertility?" *Urology*, vol. 77, no. 5, pp. 1101–1106, 2011.

[49] E. A. El Hakim, M. Epee, T. Draycott, U. D. Gordon, and V. A. Akande, "Significance of positive Chlamydia serology in women with normal-looking Fallopian tubes," *Reproductive biomedicine online*, vol. 19, no. 6, pp. 847–851, 2009.

[50] S. S. Witkin, "Immunity to heat shock proteins and pregnancy outcome," *Infectious Diseases in Obstetrics and Gynecology*, vol. 7, no. 1-2, pp. 35–38, 1999.

[51] I. Sziller, P. Fedorcsák, Z. Csapó et al., "Circulating antibodies to a conserved epitope of the *Chlamydia trachomatis* 60 kDa heat shock protein is associated with decreased spontaneous fertility rate in ectopic pregnant women treated by salpingectomy," *American Journal of Reproductive Immunology*, vol. 59, no. 2, pp. 99–104, 2008.

[52] V. Akande, C. Turner, P. Horner, A. Horne, and A. Pacey, "Impact of *Chlamydia trachomatis* in the reproductive setting: British fertility society guidelines for practice," *Human Fertility*, vol. 13, no. 3, pp. 115–125, 2010.

[53] E. Lyytikäinen, M. Kaasila, E. Hiltunen-Back et al., "A discrepancy of *Chlamydia trachomatis* incidence and prevalence trends in Finland 1983–2003," *BMC Infectious Diseases*, vol. 8, article 169, 2008.

[54] R. C. Brunham and M. L. Rekart, "The arrested immunity hypothesis and the epidemiology of chlamydia control," *Sexually Transmitted Diseases*, vol. 35, no. 1, pp. 53–54, 2008.

[55] W. L. Beatty, R. P. Morrison, and G. I. Byrne, "Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis," *Microbiological Reviews*, vol. 58, no. 4, pp. 686–699, 1994.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 407057, 6 pages
doi:10.1155/2011/407057

Research Article

Endometrial Histopathology in Patients with Laparoscopic Proven Salpingitis and HIV-1 Infection

Nelly R. Mugo,[1] Julia Kiehlbauch,[2,3,4] Nancy Kiviat,[3] Rosemary Nguti,[5] Joseph W. Gichuhi,[1,6] Walter E. Stamm,[7] and Craig R. Cohen[2,6,8]

[1] *Department of Obstetrics and Gynecology, Kenyatta National Hospital, P.O. Box 19865-00202, Nairobi, Kenya*
[2] *Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya*
[3] *Department of Pathology, Harborview Medical Center, UW Medicine Pathology, University of Washington, P.O. Box 357470, Seattle, WA 98195-7470, USA*
[4] *Maryland Department of Health and Mental Hygiene, 201 West Preston Street, Baltimore, MD 21201, USA*
[5] *Department of Statistics, University of Nairobi, Nairobi, Kenya*
[6] *Department of Obstetrics and Gynecology, University of Nairobi, School of Medicine, Kenyatta National Hospital, P.O. Box 19676, Nairobi, Kenya*
[7] *Department of Medicine, University of Washington, Seattle, WA 98195, USA*
[8] *Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, 50 Beale Street, Suite 1200, San Francisco, CA 94105, USA*

Correspondence should be addressed to Nelly R. Mugo, rwamba@csrtkenya.org

Received 18 April 2011; Revised 6 June 2011; Accepted 8 June 2011

Academic Editor: Thomas Cherpes

Study Objective. To identify sensitive and specific histological criteria for endometritis in women with laparoscopically-confirmed acute salpingitis. *Methods*. Women, age 18–40 years of age presenting with complaints of lower abdominal pain ≤2 weeks and no antibiotics use in past two weeks, were enrolled. They underwent clinical examination, screening for HIV; other sexually transmitted infections plus endometrial biopsy sampling for histopathology. Diagnostic laparoscopy confirmed the diagnosis of acute salpingitis. Controls were women undergoing tubal ligation and HIV-1 infected women asymptomatic for genital tract infection. *Results*. Of 125 women with laparoscopically-confirmed salpingitis, 38% were HIV-1 seropositive. Nineteen HIV-1 negative controls were recruited. For the diagnosis of endometritis, ≥1 plasma cells (PC) and ≥3 polymorphonuclear lymphocytes (PMN) per HPF in the endometrium had a sensitivity of 74% for HIV-1-seropositive, 63% for HIV-1-seronegative women with a specificity of 75% and positive predictive value of 85% regardless of HIV-1-infection for predicting moderate to severe salpingitis. For HIV-1-seronegative women with mild salpingitis, ≥1 PC and ≥3 PMN had a sensitivity of 16% and a PPV of 57%. *Conclusion*. Endometrial histology, did not perform well as a surrogate marker for moderate to severe salpingitis, and failed as a surrogate marker for mild salpingitis.

1. Introduction

In Africa, pelvic inflammatory disease (PID) and its sequelae are a predominant cause of gynecologic morbidity [1, 2]. These include tubal factor infertility, ectopic pregnancy, chronic pelvic pain, and recurrent pelvic infections [3, 4].

HIV-1 seroprevalence in women with PID is consistently 2–7 times greater than measured in matched populations without PID [5–7]; both infections are most commonly acquired through unprotected sexual activity. Prompt diagnosis and treatment of women with upper genital tract infections is important in reducing morbidity, but it is complicated by lack of a sensitive and specific clinical and laboratory diagnostic test. Laparoscopy is the gold standard for the diagnosis of salpingitis, but is not practical for routine clinical practice.

Endometrial histopathology is often used as a surrogate for upper genital tract infection. Kiviat et al. [8] evaluated

women with clinical PID; evidence of endometritis as defined by ≥1 plasma cell (PC) and ≥5 polymorphonuclear lymphocytes (PMN) per high-powered field (hpf) was 92% sensitive and 87% specific compared with visual findings of salpingitis determined by laparoscopy [8]. Using the same diagnostic criteria in a study of acute salpingitis in Kenya, plasma cell endometritis as defined by ≥1 PC/hpf was identified in 49% of women with salpingitis: this increased with disease severity and HIV-infection [7]. Studies on HIV-1-infected women have found an increased prevalence of plasma cell endometritis [9] even in the absence of clinical disease [10, 11].

Thus, we conducted this analysis to determine the optimum endometrial histopathological criteria for predicting salpingitis in a population with a high HIV-1 seroprevalence. We anticipate that these data will help to plan future clinical trials, increase the understanding of the pathogenesis of upper genital tract infection among HIV-1 infected women, and in certain circumstances provide a tool to confirm the clinical diagnosis of PID.

2. Materials and Methods

Study procedures have been previously detailed [12]. Briefly, between April 2000 and July 2002, women aged 18–40 admitted to Kenyatta National Hospital (KNH) acute gynecology ward with a complaint of lower abdominal/pelvic pain for 2 weeks or less plus one or more of the following signs or symptoms: temperature ≥ 38°C, dysuria, and complaint of abnormal vaginal discharge were eligible for enrollment.

After induction of anesthesia, an endometrial biopsy was obtained with a Pipelle suction curette (Unimar, Inc., Wilton, Conn). At laparoscopy, samples from peritoneal fluid, tubal ostia, and pyosalpinx/tubo-ovarian abscess (TOA) were obtained for *N. gonorrhoeae* and *C. trachomatis* PCR. Using the Jacobson and Westrom criteria [13], the severity of acute salpingitis was graded as (1) mild (tubal erythema or edema, mobile tubes, and with or without spontaneous exudate), (2) moderate (marked tubal erythema and edema, limited tubal mobility, questionable or no tubal patency, and gross exudate), and (3) severe (pyosalpinx or TOA).

We enrolled two sets of controls. The first group included HIV-1-seronegative women presenting to the KNH family planning clinic. Women desiring permanent sterilization underwent laparoscopic tubal ligation preceded by an endometrial biopsy obtained using a Pipelle suction curette. HIV-1-seropositive controls were enrolled from an HIV care and treatment clinic at the Center for Respiratory Disease Research at the Kenya Medical Research Institute. Subjects had no clinical evidence of PID. Enrollment and study procedures of the HIV-1-seropositive control group are detailed elsewhere [14]. After informed consent was obtained, an endometrial pipelle biopsy was obtained in the research clinic.

2.1. Laboratory Methods. Samples from the cervix, endometrium, fallopian tube, and abscess were examined by PCR (Roche Molecular Diagnostics, Pleasanton, Calif, USA) for *N. gonorrhoeae* and *C. trachomatis*. Endometrial specimens were fixed in 10% buffered formalin, processed, and stained with hematoxylin, eosin, and methyl green pyronin. PMNs in glands and PCs in stroma were counted per high-power field. One pathologist (NK) who was blinded to the patients' diagnosis read the slides. Serum was tested for HIV antibodies by ELISA (Detect HIV, BioChem ImmunoSystems, Montreal, Canada) with positive results confirmed by a second ELISA (Recombigen, Cambridge Biotech, Ireland).

2.2. Data Analysis. Data were analyzed using SPSS for Windows 10.0 (SPSS Inc., Chicago, USA). Univariate analyses used chi-square and Fisher's exact tests for categorical data and Student's t-test for continuous variables. Logistic regression was done for multivariate analysis.

3. Results

3.1. Description of Study Population. One hundred and sixty women were enrolled with clinical PID, 140 (88%) had laparoscopically confirmed salpingitis, 125 (89%) of whom had an endometrial biopsy specimen: 56 (45%) had mild, 31 (25%) had moderate, and 38 (30%) had severe disease based on laparoscopic criteria. Nineteen women had other diagnoses at laparoscopy including appendicular abscess ($n = 2$), endometriosis ($n = 1$), ovarian cyst ($n = 12$), frozen pelvis ($n = 1$), pelvic tuberculosis ($n = 1$), cancer of the sigmoid volvulus with abscess ($n = 1$), and ovarian torsion ($n = 1$). Asymptomatic women ($n = 20$) desiring permanent sterilization underwent laparoscopic tubal ligation and served as HIV-1-negative controls. A single control subject had a sticky exudate emanating from the Fallopian tubes and was excluded from the analysis leaving 19 HIV-1-seronegative controls. Forty-five asymptomatic HIV-1-seropositive controls were enrolled from an HIV care clinic; one woman had *C. trachomatis* detected.

Forty-eight (38%) of the women with salpingitis were HIV-seropositive. Women with salpingitis were younger, less likely to be married, and less likely to have ever used contraception (Table 1). As expected, none of the HIV-1-seronegative controls had signs or symptoms consistent with PID, and none were infected with *N. gonorrhoeae* or *C. trachomatis*. However, *T. vaginalis* was detected in a similar proportion of salpingitis cases (23%) and HIV-seronegative controls (21%) (Table 1).

3.2. Factors Associated with Evaluable and Unevaluable Endometrial Histopathology. Of the 125 women with salpingitis, endometrial biopsies from 107 (86%) were evaluated histological. Overall, 77 (72%) were adequate for histological diagnosis. Inadequate biopsies corresponded to endometrial specimens demonstrating sloughing, frank pus, and lack of tissue. In general, more severe disease as demonstrated by higher clinical severity score (CSS) (15.5 versus 13, $P < .03$) and severity of salpingitis based on laparoscopic findings (P-trend $< .04$) was associated with unevaluable endometrial biopsy results (Table 2). Similarly, history of depomedroxyprogesterone acetate (DMPA) was associated

Table 1: Comparison of demographic, clinical history and signs, and laboratory findings for women laparoscopically diagnosed with salpingitis and women undergoing tubal ligation (controls).

Variables	Salpingitis $N = 125$	HIV −/ve Controls $N = 19$	P value
Demographics and clinical history			
Age mean years (SD)	27.8 (5.5)	34.3 (4.1)	0.001
Education mean years (SD)	8.7 (2.9)	8.2 (2.5)	0.5
Marital status:			
Single	31 (25%)	0	Ref.
Married	70 (56%)	17 (90%)	0.008
Divorced/separated	20 (16%)	2 (10%)	0.12
Ever use of contraceptives:			
Oral contraceptives	55 (44%)	16 (84%)	0.001
DMPA	40 (32%)	15 (80%)	0.001
IUD	19 (15%)	8 (42%)	0.005
Condoms	48 (39%)	3 (16%)	0.04
Clinical findings			
Clinical severity score (CSS), median, (mode), range	14 (8) 32	0 (0) 5	0.00
Laboratory findings			
HIV-1	48 (38%)	0	0.001
Gonorrhea and/or chlamydia	23 (18.4)	0	0.04
Trichomonas vaginalis	23 (19%)	4 (21%)	0.8
Adequate endometrial biopsy	77 (72%)	12 (63%)	0.4

with an increased likelihood of obtaining an unevaluable biopsy ($P = .02$). HIV-infected women were more likely to have an unevaluable endometrial biopsy (57% versus 36%, $P < .05$) than HIV-uninfected women. Although not significant, participants with an inadequate endometrial histological specimen had a higher prevalence of gonorrhea compared to those with an adequate biopsy (23% versus 12% $P < 0.23$) (Table 2).

In multivariate analysis, after controlling for factors found significant in univariate analysis, the use of DMPA at any time (adjusted OR = 3.1, 95% CI 1.1–8.5), HIV-1 infection for women with mild (AOR = 4.6, 95% CI 1.1–18.3) but not moderate salpingitis (AOR = 0.89, CI 0.15–5.3), or severe salpingitis (AOR = 2.63, CI 0.68–10.2) was associated with an increased odds of an unevaluable endometrial biopsy. In addition, 12 (63%) of 19 specimens from HIV-1-seronegative subjects were evaluable for histopathology.

3.3. Distribution of PMN and PC in the Endometrial Biopsy: Effect of HIV-1 Serostatus and Disease Severity. We reviewed the distribution of PMN and PC by HIV-1 serostatus and severity of salpingitis. Women with severe salpingitis regardless of HIV-1 serostatus had the highest frequency of PMN and PC per high-power field. Only two patients with HIV-1 infection and salpingitis did not have PMN found in the endometrium. Although PMN density did not increase with severity of salpingitis among women with HIV-1 infection (P-trend = .49), this association was significant for HIV-1-uninfected women with salpingitis (P-trend = .05). In contrast, the frequency of PCs increased with severity of salpingitis among those with HIV-1 infection (P-trend = .04), but not among HIV-1 uninfected (P-trend = .14). Furthermore, HIV-1 infection was associated with a higher frequency of PCs/hpf (P-trend < .001), and presence of lymphoid follicles ($P < .04$). Only 2 (6%) of 34 HIV-1-infected women with salpingitis did not have any plasma cells present in the endometrium versus 23 (41%) of 56 HIV-1-uninfected women with salpingitis.

3.4. Comparison of Endometrial Histopathology Findings and Salpingitis. We next set out to determine the sensitivity, specificity, and positive predictive value of four histopathologic criteria for diagnosis of endometritis in comparison to the laparoscopic diagnosis of salpingitis. The four rules evaluated included: (a) ≥3 PMN and ≥1 PC per high-power field, (b) ≥1 PMN and ≥1 PC per high-power field, (c) ≥1 PMN per high-power field, and (d) ≥1 PC per high-power field. Women with moderate and severe disease were grouped together and compared to women with mild salpingitis and to the two control groups. Table 3 outlines the comparison between the laparoscopic diagnosis for mild and moderate/severe salpingitis and the four histological rules stratified by HIV-1 serostatus. Because the diagnosis of the moderate and severe disease requires more objective evidence of tubal inflammation (e.g., pus from tubes, pyosalpinx, abscess, and fresh adhesions) than mild disease, we chose to gauge the sensitivity of each histological rule using laparoscopic evidence of moderate/severe salpingitis as the "gold standard." Rule "a", although less sensitive than rules "b" through "d" for women with moderate/severe salpingitis (HIV-seropositive = 74% versus 63%; HIV-seronegative; 93% versus 75%), was the most specific, demonstrating

Table 2: Comparison of demographic, clinical history and signs, and laboratory findings for women laparoscopically diagnosed with salpingitis, with and without an endometrial biopsy adequate for histological evaluation.

Variables	Adequate biopsy $N = 77$ (72%)	Inadequate biopsy $N = 30$ (28%)	P value
Demographics and history			
Age mean (SD)	27.8 (5.5)	27.9 (6.1)	0.9
Infertility ≥ 1 year	26 (39%)	3 (13%)	0.02
Ever use of contraceptive:			
None	56 (51%)	23 (77%)	Ref
Oral contraceptives	36 (47%)	13 (43%)	0.75
DMPA	18 (23%)	14 (47%)	0.02
Intrauterine device	13 (17%)	3 (10%)	0.37
Symptoms			
Abnormal menstruation	16 (21%)	6 (21%)	1.0
Clinical examination findings			
Clinical severity score, median (mode) range			
Total clinical severity score	13 (8) 28	15.5 (4) 30	0.03
Laparoscopic salpingitis severity			
Mild	32 (42%)	15 (50%)	
Moderate	26 (36%)	3 (10%)	
Severe	19 (25%)	12 (40%)	0.04
Pelvic Abscess	13 (17%)	9 (30%)	0.15
Laboratory Findings			
HIV-1	27 (36%)	17 (57%)	0.05
CD4 count < 200/μL	8 (11%)	7 (23.3%)	0.09
White cell count	9.7 (6.04)	10.3 (5.5)	0.63
Lymphocytes (blood) %	26.9 (13.4)	19.1 (7.8)	0.001
Gonorrhea and/or chlamydia	11 (14%)	8 (27%)	0.13
Gonorrhea	9 (12%)	7 (23%)	0.23
Chlamydia	3 (4%)	1 (3%)	0.9
Trichomonas vaginalis	14 (19%)	6 (21%)	0.8
Bacterial vaginosis (Gram's stain)	30 (45%)	16 (62%)	0.2

endometritis in 25% and 7% of HIV-1-seronegative and HIV-1-seropositive controls, respectively, in comparison to 58%–67% and 38%–62% for rules "b" through "d". Among the 19 women enrolled with a clinical diagnosis of PID, but who did not have salpingitis on laparoscopy, rule "a" had the least false positive, while rule "d", at least one plasma cell, scored the highest false-positive rate.

4. Discussion

This study had three key findings: (1) ≥3 PMN and ≥1 PC per hpf as a histologic criteria for the diagnosis of moderate to severe salpingitis, while performing better than the other criteria, appears to have limited utility even more so for cases of mild salpingitis; (2) endometrial specimens were often unevaluable for histopathology, and unevaluable specimens were more likely in subjects with severe salpingitis and HIV-infection, and thus may affect the utility of endometrial histopathology to confirm the clinical diagnosis of PID in similar settings; (3) The PMN response increased with disease severity for HIV-1 seronegative but not HIV-1 seropositive women with salpingitis.

Since Kiviat et al. published their paper, [8] histologic endometritis has been used as a surrogate marker for salpingitis, especially in the study of mild to moderate PID. Even though the criteria for histologic endometritis had never been validated in HIV-1-infected populations, several studies of PID were conducted in high HIV-1 seroprevalence settings [6, 7, 9, 12, 14]. The results of this study did not validate the Kiviat et al. 1990, criteria for ≥5 PMNs and ≥1 PC for the diagnosis of PID. In the Kiviat et al. cohort, *N. gonorrhoeae* and/or *C. trachomatis* was found in 49% of the patient population; in comparison, the cohort in our study had a high HIV-1 prevalence and a combined gonorrhea and/or chlamydia prevalence of 18% (Table 1).

Similar to another report, only 72% of endometrial biopsies in our study were evaluable [1]. The increased frequency of unevaluable endometrial biopsies in women with severe salpingitis, likely due to increased endometrial sloughing and presence of pus, and HIV-1 infection further limits the utility

Table 3: Presence and density of polymorphonuclear leucocytes (PMN) and plasma cells (PC) on histopathology of endometrial biopsies in women with mild, moderate, and severe salpingitis at laparoscopy and controls in HIV-seropositive and -seronegative women.

Rules representing cell/hpf	Mild salpingitis $N = 32$		Moderate/severe Salpingitis $N = 50$		Controls without salpingitis $N = 62$	
	HIV +/ve ($N = 7$)	HIV −/ve ($N = 25$)	HIV +/ve ($N = 23$)	HIV −/ve ($N = 27$)	HIV +/ve ($N = 45$)	HIV −/ve ($N = 12$)
≥3 PMN and ≥1 PC	4 (57%)	4 (16%)	17 (74%)	17 (63%)	3 (7%)	3 (25%)
≥1 PMN and ≥1 PC	7 (100%)	8 (32%)	19 (83%)	20 (74%)	17 (38%)	7 (58%)
≥1 PMN	7 (100%)	17 (68%)	21 (91%)	21 (78%)	32 (71%)	8 (67%)
≥1 PC	7 (100%)	9 (36%)	21 (91%)	21 (78%)	28 (62%)	8 (67%)

of endometrial histopathology as a diagnostic tool for studies of PID in similar populations.

The low sensitivity of histologic endometritis for mild salpingitis amongst women symptomatic for PID was unexpected. Studies of endometritis in populations of asymptomatic women have consistently demonstrated a relatively high prevalence of endometritis [10, 15, 16] which led authors to describe endometritis as an intermediate infection to PID. Eckert et al. studied HIV-1-infected women presenting to a family planning clinic and found endometritis in 38% of participants [10]. This is a higher prevalence than what we found in HIV-1-negative women (16%) and a lower prevalence than what we found in HIV-1-seropositive women (57%) with mild salpingitis using less stringent criteria for endometritis. Furthermore, a prior laparoscopic study demonstrated salpingitis in the absence of endometritis [7]. An alternative explanation may result from the subjectivity of the laparoscopic criteria for mild salpingitis that leads to misclassification of cases [13].

The distribution of PMNs and PCs in the endometrium of women with salpingitis was affected by HIV-1 serostatus and disease severity. PMNs are only found in the healthy endometrium during menses [17], and form part of the endometrial immune response, they are also the first line immune defense against bacterial infections. The increased density of PMN with severe disease in HIV-1-uninfected but not in HIV-1-infected women with salpingitis is not well understood. Consistent with other studies [7, 9, 10, 14], we found increased PC endometritis with HIV-1 infection. This could represent HIV-1 infection in the genital tract [18]; chronic plasma cell endometritis [11, 14]; or the presence of opportunistic infections. Cherpes et al. reported an association between HSV-2 seropositivity and plasma cell endometritis [19]; notably HSV-2 is extremely prevalent among HIV-1-infected persons (KAIS 2007 [20]. Contrary to these findings, Eckert et al. [10] evaluated 20 endometrial biopsy samples from women with asymptomatic histologic endometritis and failed to detect herpes simplex virus by PCR, and cytomegalovirus was detected equally in women with and without histological endometritis. *Mycoplasma genitalium* is another potential cause of endometritis [21].

One limitation of this study is that HIV-1-infected controls did not undergo laparoscopic evaluation. Therefore, unlikely we cannot firmly exclude subclinical salpingitis from this population as we can for the HIV-1-seronegative controls. Furthermore, we did not attempt to detect suspected etiologies of endometritis such as bacteria other than *N. gonorrhoeae* and *C. trachomatis* including *M. genitalium* [14] and potential etiologies such as cytomegalovirus and herpes simplex virus infection. Such data might help to elucidate the reason for the different findings among HIV-1-seropositive and HIV-1-seronegative women with salpingitis in regards to endometrial histopathology.

This study raises some important questions regarding PID and its sequelae. With increased access to highly active antiretroviral therapy (HAART), HIV-1-infected women are living longer. Population data from Uganda [22] plus others [23] have demonstrated reduced fertility in HIV-1-infected women regardless of disease stage. It is plausible that PC endometritis may lead to reduced fertility. Further research is required to determine if women using HAART return to normal fertility or not. Lastly, although endometrial histopathology serves as a reasonable surrogate for salpingitis in HIV-1-uninfected populations, its utility in populations with a high HIV-1 seroprevalence appears to be limited. Discovery of a sensitive and specific biomarker or set of biomarkers for salpingitis could facilitate further research on PID and its sequelae in such settings.

References

[1] T. Mathews, J. K. G. Mati, and J. N. Fomulu, "A study of infertility in Kenya: results of investigation of the infertile couple in Nairobi," *East African Medical Journal*, vol. 58, no. 4, pp. 288–297, 1981.

[2] S. M. Walton and J. K.G. Mati, "An evaluation of secondary infertility in Kenya," *East African Medical Journal*, vol. 53, no. 6, pp. 310–314, 1976.

[3] L. Westrom, R. Joesoef, G. Reynolds, A. Hagdu, and S. E. Thompson, "Pelvic inflammatory disease and fertility: a cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results," *Sexually Transmitted Diseases*, vol. 19, no. 4, pp. 185–192, 1992.

[4] L. Westrom, "Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrial countries," *American Journal of Obstetrics and Gynecology*, vol. 138, no. 7, pp. 880–892, 1980.

[5] A. W. Ojwang, V. M. Lema, and S. H. M. Wanjala, "HIV infection among patients with acute pelvic inflammatory

disease at the Kenyatta National Hospital, Nairobi, Kenya," *East African Medical Journal*, vol. 70, no. 8, pp. 506–511, 1993.

[6] M. C. Kamenga, K. M. De Cock, M. E. St Louis et al., "The impact of human immunodeficiency virus infection on pelvic inflammatory disease: a case-control study in Abidjan, Ivory Coast," *American Journal of Obstetrics and Gynecology*, vol. 172, no. 3, pp. 919–925, 1995.

[7] C. R. Cohen, "Effect of human immunodeficiency virus type 1 infection upon acute salpingitis: a laparoscopic study," *Journal of Infectious Diseases*, vol. 178, no. 5, pp. 1352–1358, 1998.

[8] N. B. Kiviat, P. Wolner-Hanssen, D. A. Eschenbach et al., "Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis," *American Journal of Surgical Pathology*, vol. 14, no. 2, pp. 167–175, 1990.

[9] E. A. Bukusi, C. R. Cohen, C. E. Stevens et al., "Effects of human immunodeficiency virus 1 infection on microbial origins of pelvic inflammatory disease and on efficacy of ambulatory oral therapy," *American Journal of Obstetrics and Gynecology*, vol. 181, no. 6, pp. 1374–1381, 1999.

[10] L. O. Eckert, D. H. Watts, S. S. Thwin, N. Kiviat, K. J. Agnew, and D. A. Eschenbach, "Histologic endometritis in asymptomatic human immunodeficiency virus-infected women: characterization and effect of antimicrobial therapy," *Obstetrics and Gynecology*, vol. 102, no. 5, pp. 962–969, 2003.

[11] J. A. Kerr-Layton, C. A. Stamm, L. S. Peterson, and J. A. McGregor, "Chronic plasma cell endometritis in hysterectomy specimens of HIV-infected women: a retrospective analysis," *Infectious Diseases in Obstetrics and Gynecology*, vol. 6, no. 4, pp. 186–190, 1998.

[12] N. R. Mugo, J. A. Kiehlbauch, R. Nguti et al., "Effect of human immunodeficiency virus-1 infection on treatment outcome of acute salpingitis," *Obstetrics and Gynecology*, vol. 107, no. 4, pp. 807–812, 2006.

[13] L. Jacobson and L. Weström, "Objectivized diagnosis of acute pelvic inflammatory disease. Diagnostic and prognostic value of routine laparoscopy," *American Journal of Obstetrics and Gynecology*, vol. 105, no. 7, pp. 1088–1098, 1969.

[14] J. S. Coleman, J. Hitti, E. A. Bukusi et al., "Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts," *AIDS*, vol. 21, no. 6, pp. 755–759, 2007.

[15] A. P. Korn, N. Hessol, N. Padian et al., "Commonly used diagnostic criteria for pelvic inflammatory disease have poor sensitivity for plasma cell endometritis," *Sexually Transmitted Diseases*, vol. 22, no. 6, pp. 335–341, 1995.

[16] L. O. Eckert, S. E. Hawes, P. K. Wölner-Hanssen et al., "Endometritis: the clinical-pathologic syndrome," *American Journal of Obstetrics and Gynecology*, vol. 186, no. 4, pp. 690–695, 2002.

[17] C. Poropatich, M. Rojas, and S. G. Silverberg, "Polymorphonuclear leukocytes in the endometrium during the normal menstrual cycle," *International Journal of Gynecological Pathology*, vol. 6, no. 3, pp. 230–234, 1987.

[18] A. L. Howell, R. D. Edkins, S. E. Rier et al., "Human immunodeficiency virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract," *Journal of Virology*, vol. 71, no. 5, pp. 3498–3506, 1997.

[19] T. L. Cherpes, H. C. Wiesenfeld, M. A. Melan et al., "The associations between pelvic inflammatory disease, Trichomonas vaginalis infection, and positive herpes simplex virus type 2 serology," *Sexually Transmitted Diseases*, vol. 33, no. 12, pp. 747–752, 2006.

[20] N. R. Mugo, S. Dadabhai, K. Sabin et al., "National seroprevalence of herpes simplex virus type 2 and co-infection with HIV-1: results of the 2007 Kenya AIDS indicator survey," in *International AIDS Conference (IAS)*, 2009, poster no. MOPEB016.

[21] C. R. Cohen, L. E. Manhart, E. A. Bukusi et al., "Association between Mycoplasma genitalium and acute endometritis," *The Lancet*, vol. 359, no. 9308, pp. 765–766, 2002.

[22] R. H. Gray, M. J. Wawer, D. Serwadda et al., "Population-based study of fertility in women with HIV-1 infection in Uganda," *The Lancet*, vol. 351, no. 9096, pp. 98–103, 1998.

[23] A. Ross, D. Morgan, R. Lubega, L. M. Carpenter, B. Mayanja, and J. A. G. Whitworth, "Reduced fertility associated with HIV: the contribution of pre-existing subfertility," *AIDS*, vol. 13, no. 15, pp. 2133–2141, 1999.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 747059, 4 pages
doi:10.1155/2011/747059

Case Report

Abdominal-Pelvic Actinomycosis Mimicking Malignant Neoplasm

Teresa Pusiol,[1] Doriana Morichetti,[2] Corrado Pedrazzani,[3] and Francesco Ricci[3]

[1] *Section of Cytopathology, Institute of Anatomic Pathology, Rovereto Hospital, 38068 Rovereto, Italy*
[2] *Institute of Anatomic Pathology, Rovereto Hospital, 38068 Rovereto, Italy*
[3] *Division of Surgery, Rovereto Hospital, 38068 Rovereto, Italy*

Correspondence should be addressed to Teresa Pusiol, teresa.pusiol@apss.tn.it

Received 3 June 2011; Accepted 27 June 2011

Academic Editor: Thomas Cherpes

Abdominal-pelvic actinomycosis is often mistaken for other conditions, presenting a preoperative diagnostic challenge. In a 46-year-old female, computed tomography showed an abdominal-pelvic retroperitoneal mass extending from the lower pole of the right kidney to the lower pelvis. The patient had a 3-year history of intrauterine device. The mass appeared to involve the ascending colon, cecum, distal ileum, right Fallopian tube and ovary, and ureter anteriorly and the psoas muscle posteriorly. The resection of retroperitoneal mass, distal ileum appendicectomy, right hemicolectomy, and right salpingo-oophorectomy was performed. The postoperative period was uneventful. Penicillin therapy was given for six months without any complication. The retroperitoneal mass measured $4.5 \times 3.5 \times 3$ cm, surrounded adjacent organs and histologically showed inflammatory granulomatous tissue, agglomeration of filaments, and sulfur granules of *Actinomyces*, with positive reaction with periodic acid Schiff. Right tubo-ovarian abscess was present. Abdominalpelvic actinomycosis should always be considered in patients with a pelvic mass especially in ones using intrauterine device.

1. Introduction

In developed countries, actinomycosis is a relatively rare disease that is mainly caused by *Actinomyces israelii*. *Actinomyces israelii* is an anaerobic, gram-positive organism that is normally present in oral cavity, throughout the gastrointestinal tract, female genital tract, and the bronchus. Actinomycosis occurs most frequently in the cervical facial (50%–65%), abdominal (20%), and thoracic (15%) regions. The overall incidence of registered cases of actinomycosis is decreasing. Abdominalpelvic actinomycosis, however, are increasing in frequency and is associated with abdominal surgery (such as appendectomy), bowel perforation, or trauma [1]. In addition, the presence of a long-standing intrauterine device (IUD) is a reported risk factor in young women [2]. The abdomen is the most frequent site for actinomycosis and when an abdominal tumor presents as the clinical symptom, the local lesion needs to be differentiated from abdominal tumors of other etiologies, malignancy in particular.

In the majority of cases, the indolent clinical course together with the malignant like tumour appearance at imaging investigations make a delay in diagnosis the rule rather than the exception. Preoperative diagnosis is usually difficult with the majority of cases being diagnosed after the histological and bacteriological examination of the resected specimen. The present paper discusses the case of an abdominalpelvic actinomycosis mimicking a malignant retroperitoneal tumour in a young insulin-dependent diabetic Italian woman with 3-year history of IUD.

2. Case Presentation

A 46-year-old female was referred to our unit following a computed tomography (CT) scan which demonstrated an abdominalpelvic retroperitoneal mass. The patient had came to emergency department complaining of a three-day history of a lump on the right lower limb preceded by fever and continuous right lower abdominal pain irradiated to the back for the previous 3 weeks. Past medical history was unremarkable except for insulin-dependent diabetes mellitus since 11 years of age. The patient had a 3-year history of IUD which had recently been removed. Physical examination demonstrated mild oedema of the right leg, with no abdominal abnormal findings. Doppler ultrasonography of the lower

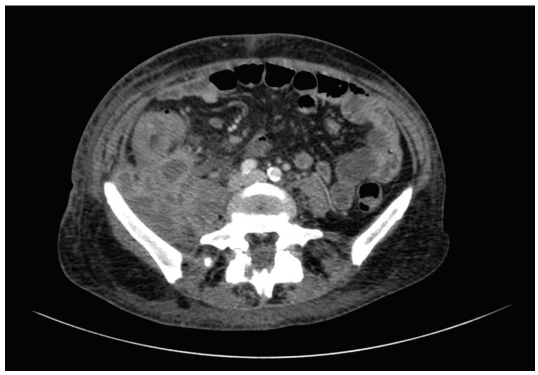


Figure 1: Computed tomography (CT) scan of the abdomen showed the mass occupying the retroperitoneal space and infiltrating the ascending colon.

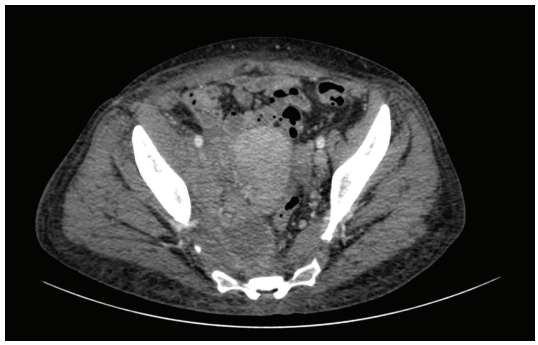


Figure 2: Computed tomography (CT) scan showed the mass occupying the extraperitoneal space infiltrating the psoas muscle posteriorly and the pelvis anteriorly.

limbs was carried out and ruled deep venous thrombosis and superficial thrombophlebitis. The patient was discharged and she was investigated as an outpatient. Biochemical and haematological investigations demonstrated a raised CRP and ESR, normal white blood count, mild macrocytic anemia (Hb 7.9 g/dL, MCV 100 fL), and thrombocytosis (PLT 626.000/uL). The CT scan showed a retroperitoneal mass with abscess areas and necrosis extending from the lower pole of the right kidney to the lower pelvis. The mass appeared to involve the ascending colon, cecum, distal ileum, right Fallopian tube and ovary, and ureter anteriorly and the psoas muscle posteriorly (Figures 1 and 2). Right ureteric dilatation was evident. A colonoscopy was carried out to investigate the possibility of inflammatory bowel disease or a colonic perforated cancer. The endoscopic examination was normal except for the presence of nonspecific mucosal inflammation of the distal ileum. A US-guided fine needle aspiration biopsy of the mass was hence performed. The cytological specimen showed inflammatory cells, with no evidence of malignant cells. Tuberculous and nontuberculous mycobacterium DNA was also negative.

The patient was hence referred to surgery division in the suspect of malignant retroperitoneal mass.

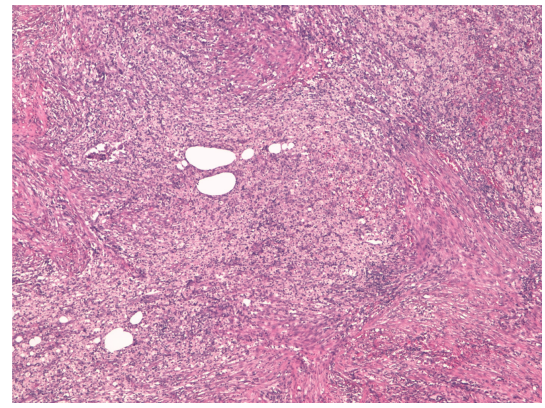


Figure 3: The retroperitoneal mass consisted of chronic suppurative granolomatous inflammation, H&E 20x.

A right ureteric stent was placed and an explorative laparotomy was preformed. The intraoperative findings were compatible with a neoplastic mass originating from the retroperitoneum. Debulking of retroperitoneal, appendicectomy right hemicolectomy extended to the distal ileum, and right salpingo-oophorectomy were performed. The postoperative period was uneventful and the patient was discharged in postoperative day 9.

Penicillin therapy was given for six months without any complication. She is well and has gained weight after one year.

The retroperitoneal mass measured $4.5 \times 3.5 \times 3$ cm, surrounded adjacent organs and histologically showed inflammatory granulomatous tissue composed by granulocytes, fibroblasts, xanthomatous cells, and agglomeration of filaments and sulfur granules of *Actinomyces*, with positive reaction with periodic acid-Schiff and Grocott's dye. Abscess formation, necrosis were found (Figures 3, 4, 5 and 6). Similar inflammatory granulomatous process was present in the serosa of terminal ileum, appendix, cecum, ascending colon with extension to corresponding mesentery. Regional 22 lymph nodes were free of disease. Right tubo-ovarian abscess was present. The mucosa of all organs examined did not show actinomycosis but only congestion and slight aspecific inflammation.

3. Discussion

Actinomyces israelii as other bacteria of the *Actinomyces* species are saprophytes in the oral cavity, gastrointestinal, and female genital tract. The destruction of the muscular barrier by trauma, that is, endoscopic manipulation, operations, immunosuppression, and chronic inflammatory disease, is recognized as predisposing factors for penetration of *Actinomyces* bacteria [3]. Several forms of immunosuppression, such as leukemia, lymphoma, renal insufficiency, renal transplant, and diabetes, have been demonstrated to facilitate this process [4]. It is accepted that the risk of pelvic actinomycosis resulting from IUD use is very low. Only about 92 reported cases exist in the published English language literature,

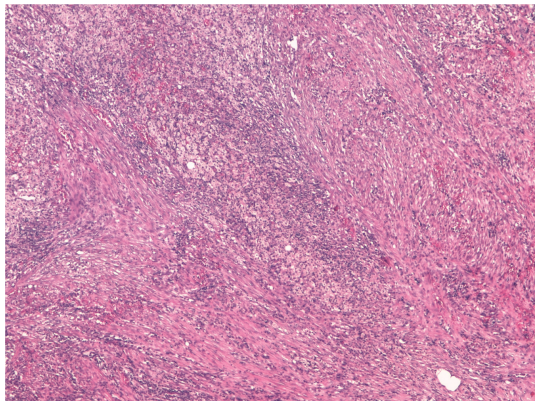


Figure 4: The inflammation was composed by fibroblasts, xanthomatous cells, and neutrophilic granulocytes, H&E 20x.

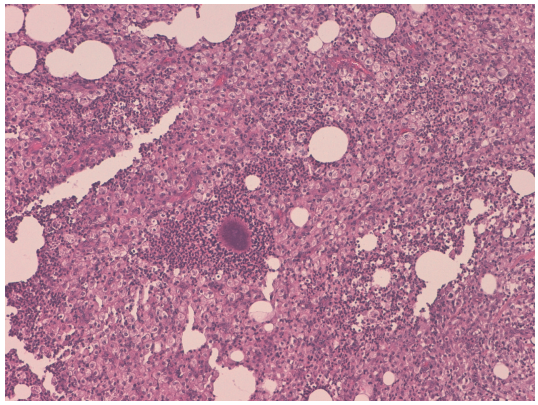


Figure 5: Colonies of *Actinomyces* species were detected with surrounding inflammatory infiltration, H&E 40x.

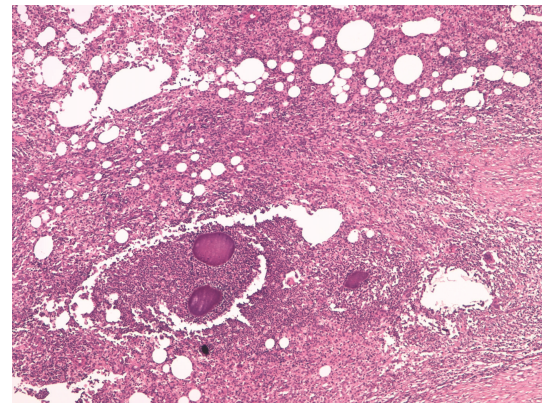


Figure 6: Colonies of *Actinomyces* species were founded in abscess areas, H&E 40x.

despite 30 million patient-year of IUD use [5]. About 80% of cases of pelvic actinomycosis have been reported in women using an IUD. *Actinomyces israelii* infects 1.65% to 11.6% of IUD users, and infection is more common in women who have had an IUD use in situ longer than four years [6].

Our patient had a 3-year history of IUD which had recently been removed. The IUD may be considered the initial trigger of abdominalpelvic actinomycosis. Ileocecal region and appendix itself are the most frequently involved regions. Recognized causes of infection are appendicitis, diverticulitis, inflammatory bowel disease, and previous open and laparoscopic surgery. Endoscopic procedures have been also described as rare potential causes. No previous surgery or history of inflammatory diseases of the abdomen were reported by our patient.

Clinical symptoms are usually not specific and include a wide range of clinical presentation. Acute abdomen can be observed when complications such as perforation or fistulization occur; more frequently, as in our case, abdominal pain is present.

Preoperative diagnosis of pelvic abdominal actinomycosis can be difficult because of the insidious nature of the infection. Biochemical and haematological investigations are almost not specific. Usually, diagnosis with fine-needle aspiration cytology is in impossible pre-operatively. In fact the filaments and sulfur granules of *Actinomyces* are surrounded by extensive inflammatory tissue that is the sample site of fine-needle aspiration cytology. In our case these procedures were conclusive of inflammatory lesion.

Preoperative radiologic diagnosis is rarely performed. Ha et al. [7] analyzed the CT findings of ten patients with abdominal actinomycosis. The aggressive nature of invasion and infiltration of contiguous tissues and organs, such as the large intestine, greater omentum, or abdominal wall, was remarkable and comparable to that seen in acute necrotizing pancreatitis. Lee et al. [8] have examined CT scans in 18 patients with pathologically proved abdominalpelvic actinomycosis involving the gastrointestinal tract. Eight patients had a history of using IUDs. The sigmoid colon was most commonly involved (50%). All patients showed concentric ($n = 15$) or eccentric ($n = 3$) bowel wall thickening, with a mean thickness of 1.2 cm and a mean length of 8.3 cm. The thickened bowel enhanced homogeneously in nine patients and heterogeneously in the other nine. Inflammatory infiltration was mostly diffuse and severe. In 17 patients, a peritoneal or pelvic mass (mean maximum diameter, 3.2 cm) was seen adjacent to the involved bowel and appeared to be heterogeneously enhanced in most cases; infiltration into the abdominal wall was seen in four patients.

Actinomycosis should be included in the differential diagnosis when CT scans show bowel wall thickening and regional pelvic or peritoneal mass with extensive infiltration, especially in patients with abdominal pain, fever, leukocytosis, or long-term use of intrauterine contraceptive devices.

Neoplasms and other inflammatory diseases, especially tuberculosis or Crohn's disease, may be confused with actinomycosis. In actinomycosis, solid masses with focal low-attenuation areas were more frequently found than cystic masses with thickened walls. In conclusion, imaging investigations (US, CT, and MRI) confirm the presence of a mass with collections but they are not able to distinguish between actinomycosis and malignancy, Crohn's disease, diverticulitis, appendicitis, pelvic peritonitis, or tubercolosis [9].

The infiltrative mass with unusual aggressiveness is the one of important radiological findings.

In our case the CT scan showed an infiltrative mass with unusual aggressiveness. The lymph node enlargement, ascites and involvement of the whole peritoneal cavity were absent. These findings could be supported by the diagnosis of Actinomycosis in our case.

Similarly to our case, in the great majority of cases, diagnosis is reached by histopathological examination of the specimen obtained by surgical exploration and resection. Histopathologic examination of the infected tissue should include a search for characteristic, but not pathognomonic, appearances of sulphur granules. The granules measure 0.4–4 mm and stain Gram-positive with a mycelium-like structure [10]. The differential diagnosis of sulphur granules, however, includes nocardiosis, streptomycosis, chromomycosis, eumycetoma, and botryomycosis [11]. *Actinomyces* granules regularly show a positive reaction with periodic acid Schiff and Grocott's dye, but the Kossa reaction is negative. Pseudoactinomyces granules formed by *Nocardia* and *Streptomyces* spp. show the opposite reactions [12]. Because of the size of the bacterium, it usually does not spread via the lymphatic system; therefore, regional lymphadenopathy is uncommon or develops late [13]. In our case the intense proliferation of fibroblasts and xanthomatous cells may be considered the cause of sizes of retroperitoneal mass simulating malignancy. The necrosis and abscess areas have progressively increased the inflammatory mass with compression and infiltration of adjacent organs. The histological examination showed regional lymph nodes free of disease.

4. Conclusions

The primary diagnosis of abdominalpelvic actinomycosis is difficult. The clinical picture has changed in the last ten years. Women with IUDs are especially at risk. All organs and anatomic structures of the abdomen can be involved. Even with extensive infection, combined operative and antibiotic therapy allows cure in most cases.

References

[1] J. F. Yeguez, S. Martinez, L. R. Sands, and M. D. Hellinger, "Pelvic actinomycosis presenting as malignant large bowel obstruction: a case report and a review of the literature," *The American Journal of Surgery*, vol. 66, no. 1, pp. 85–90, 2000.

[2] B. Filipovic, N. Milinic, G. Nikolic, and T. Ranthelovic, "Primary actinomycosis of the anterior abdominal wall: case report and review of the literature," *Journal of Gastroenterology and Hepatology*, vol. 20, no. 1, pp. 517–520, 2005.

[3] U. Kodali, R. Mallavarapu, and M. J. Goldberg, "Abdominal actinomycosis presenting as lower gastrointestinal bleeding," *Endoscopy*, vol. 35, no. 5, pp. 451–453, 2003.

[4] D. C. Dominguez and S. J. Antony, "Actinomyces and nocardia infections in immunocompromised and non immunocompromised patients," *Journal of the National Medical Association*, vol. 91, no. 1, pp. 35–39, 1999.

[5] A. S. Fiorino, "Intrauterine contraceptive device-associated actinomycotic abscess and actinomyces detection on cervical smear," *Obstetrics and Gynecology*, vol. 87, no. 1, pp. 142–149, 1996.

[6] J. F. McCormick and R. D. F. Scorgie, "Unilateral tubo-ovarian actinomycosis in the presence of an intrauterine device," *The American Journal of Clinical Pathology*, vol. 68, no. 5, pp. 622–627, 1977.

[7] H. K. Ha, H. J. Lee, H. Kim et al., "Abdominal actinomycosis: CT findings in 10 patients," *The American Journal of Roentgenology*, vol. 161, no. 4, pp. 791–794, 1993.

[8] I. J. Lee, H. K. Ha, C. M. Park et al., "Abdominalpelvic actinomycosis involving the gastrointestinal tract: CT features," *Radiology*, vol. 220, no. 1, pp. 76–80, 2001.

[9] P. Acquaro, F. Tagliabue, G. Confalonieri, P. Faccioli, and M. Costa, "Abdominal wall actinomycosis simulating a malignant neoplasm: case report and review of the literature," *The World Journal of Gastrointestinal Surgery*, vol. 2, no. 7, pp. 247–250, 2010.

[10] C. Jung, J. Wagner, B. Heine, J. Sieper, A. Distler, and J. Braun, "Abdominelle aktinomykose nach magenoperation bei einer patientin mit langjähringer rheumatoider arthritis unter methotrexat-therapie," *Deutsche Medizinische Wochenschrift*, vol. 124, no. 37, pp. 1066–1070, 1999.

[11] A. H. Ormsby, T. W. Bauer, and G. S. Hall, "Actinomycosis of the cholecystic duct: case report and review," *Pathology*, vol. 30, no. 1, pp. 65–67, 1998.

[12] S. Kamprath, A. Merker, R. Kühne-Heid, and A. Schneider, "Abdominale aktinomykose bei IUD," *Zentralbl Gynakol Journal*, vol. 119, no. 1, pp. 21–24, 1997.

[13] D. F. Bennhoff, "Actinomycosis: diagnostic and therapeutic considerations and a review of 32 cases," *Laryngoscope*, vol. 94, no. 9, pp. 1198–1217, 1984.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 675360, 7 pages
doi:10.1155/2011/675360

Review Article

Nonhuman Primate Models Used to Study Pelvic Inflammatory Disease Caused by *Chlamydia trachomatis*

Jason D. Bell,[1] Ingrid L. Bergin,[2] Kelsey Schmidt,[3] Melissa K. Zochowski,[1] David M. Aronoff,[4] and Dorothy L. Patton[5]

[1] *Department of Obstetrics & Gynecology, University of Michigan, 1500 East Medical Center Drive, L4510, Ann Arbor, MI 48109, USA*
[2] *Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI 48109, USA*
[3] *College of Literature, Science, and the Arts, University of Michigan, Ann Arbor, MI 48109, USA*
[4] *Reproductive Sciences Program and the Division of Infectious Diseases, Department of Internal Medicine, Department of Microbiology & Immunology, University of Michigan, Ann Arbor, MI 48109, USA*
[5] *Department of Obstetrics & Gynecology, University of Washington, Seattle, WA 98195, USA*

Correspondence should be addressed to Jason D. Bell, jasonbel@umich.edu

Received 15 May 2011; Accepted 3 June 2011

Academic Editor: Thomas Cherpes

Pelvic inflammatory disease (PID) is a global health concern that is associated with significant morbidity and is a major cause of infertility. Throughout history animals have been used for anatomical studies and later as models of human disease. In particular, nonhuman primates (NHPs) have permitted investigations of human disease in a biologically, physiologically, and anatomically similar system. The use of NHPs as human PID models has led to a greater understanding of the primary microorganisms that cause disease (e.g., *Chlamydia trachomatis and Neisseria gonorroheae*), the pathogenesis of infection and its complications, and the treatment of people with PID. This paper explores historical and contemporary aspects of NHP modeling of chlamydial PID, with an emphasis on advantages and limitations of this approach and future directions for this research.

1. Introduction

The Center for Disease Control and Prevention approximates that 750,000 women in the United States contract pelvic inflammatory disease (PID) each year and 10% of these cases result in infertility [1]. It is the ascent of bacterial organisms to the upper reproductive tract that causes PID. The majority of PID cases are due to sexually transmitted infections with *Chlamydia trachomatis* and *Neisseria gonorroheae*. *C. trachomatis* is a common cause of PID in women and is usually an asymptomatic infection, which allows the organism to remain undetected as it ascends into the upper reproductive tract [2]. High rates of infertility and morbidity resulting from PID are a global concern [3].

Nonhuman primates (NHPs) have a long history of use in biomedical research. In the first century A.D., Barbary apes (*Macaca sylvan*) were likely the first primates used in research [4]. These apes were used as replacements for human cadavers in anatomical studies. In the late 19th century, recognition of the similarities between humans and NHPs promoted the usefulness of these animals in research [5, 6]. In the last hundred years the primate has become a common research subject in many United States based laboratories. As this interest evolved, more primate species were used in biomedical research. Originally, primates were used strictly as anatomical models, but this evolved to include studies of disease pathogenesis and the testing of novel medical technologies. The similarities between the anatomy and physiology of NHPs and humans have made such modeling successful [7–9]. The specific utilization of NHPs in reproductive studies has occurred more recently and continues to expand.

In 1950, Te Linde and Scott noted the effectiveness of using NHPs (rhesus monkey) in the study of endometriosis [10]. The anatomical and physiologic similarities of NHPs permitted the modeling of diseases that would otherwise be impossible, or unethical, in humans. Because no single

species of NHP entirely reproduces the pathophysiology of human disease, various NHPs (marmoset, macaque, grivet, and baboon) have been used. NHP models of sexually transmitted infections have permitted a better understanding of disease pathogenesis, the effectiveness of different treatment modalities and have accelerated renewed research interests in prevention strategies, including topical microbicide and vaccine development. NHP models of PID, particularly *C. trachomatis*-associated PID, have been used for over five decades. *C. trachomatis* has been used to successfully infect a variety of NHPs: chimpanzees, marmosets, grivets, macaques, and baboons [11–15]. Primates provide an attractive model for PID because, unlike other animal models (sheep, guinea pigs, pigs, and mice), NHP reproductive tracts are of a similar shape and size to those of women [16]. What is more, contemporary NHP PID models employ the use of human serovars of *C. trachomatis* to induce PID, as they are naturally susceptible to infection of the lower and upper genital tract after challenge. When smaller animal models are used nonhuman strains, exogenous pretreatment with hormones as well as use of PID-inducing pathogens often need to be employed because human-derived bacterial strains may not cause a similar disease in the particular animal [17, 18]. This paper presents the current status of NHP models for the study of experimentally induced chlamydial PID.

2. The Rationale for NHP Use in Biomedical Research

In theory, the best model for human disease is the human; however ethical and practical concerns prohibit many fundamental studies. Thus, NHPs serve a unique niche in biomedical research. The evolutionary closeness that the NHPs share with *Homo sapiens* helps ensure biologically similar models with both predictive and discriminative abilities unavailable in other animal models [19]. Though research performed in rodents, and other small animals has several advantages: lower cost, ease of handling, and the ability to genetically alter them (*e.g.,* knockout mice), the results are not always translatable to human disease.

The use of NHPs in PID models has been able to successfully fulfill Koch's postulates to prove that *Chlamydia* causes PID [12, 14, 20, 21]. NHP models allow for detailed observations of disease progression, that is, the pathogenesis of infection, to be studied *in vivo*. Likewise, one can effectively examine host immune and physiological responses independently from studying microbial pathogenesis [22]. While these advantages are shared with other animals, a unique advantage of NHPs is their relatively long life span that permits animals to be reused in subsequent independent studies during their lifetime. In addition, many NHPs continue their menstrual cycle while in captivity, supporting their use as reproductive health models. Differences both subtle and great have led to the creation of PID models among different species (Table 1).

Though NHPs have numerous advantages they are not without limitations. Using NHPs is costly, requires adequate facilities, veterinarian staff support, expertise, and technical staff to care for the animals. And, due to the increasing debate regarding vivisection, adequate security is imperative.

3. Early NHP Studies of *Chlamydia* Pathogenesis

Thygeson, an ophthalmologist, studied ocular chlamydial infections and reported that maternal-to-infant transmission induced chlamydial cervicitis (Table 2) [11]. Using material collected from the eye of an infant with inclusion blennorrhea the cervix of two baboons were directly inoculated. One animal developed a marked cervicitis with purulent material in the cervical canal at 12 days postinoculation [11]. Thesecond animal had no response after the first inoculation. With a repeated inoculation this animal developed mild cervicitis but without a purulent discharge. Interestingly, Thygeson and his colleagues were unable to demonstrate the detection of inclusion bodies after inoculation in either animal.

Alexander and colleagues used the Taiwan monkey in a similar fashion, inoculating the cervix of pregnant monkeys with TRIC (Table 2) [31]. Like Thygeson, they reported mild erythema of the lower genital tract and were unable to detect inclusion bodies after inoculation. These early studies were not designed to model PID and had limited results; but they provided important foundation for later use of NHPs in PID modeling.

4. Early NHP Models of Lower Genital Tract *Chlamydia* Infection

Johnson et al. used the marmoset to show that an acute inflammatory response reaction occurred with a vaginal inoculation of *C. trachomatis* (Table 2) [20]. This response lasted 10–42 days. Acute cervicitis was characterized by erythema, occasional edema, and the presence of purulent cervical mucus [35]. A neutrophil-rich leukocyte response was shown, and chlamydial inclusions were demonstrated in the epithelium. The lower genital tract response was characterized using histology and colposcopy. Interestingly, animals with repeated infection, with either homologous or heterologous chlamydial strains, had decreased inflammatory responses, shorter durations of infection and those that had previous inoculations were able to eliminate the infection within one week [34].

Nearly all animals in their study developed either an IgM or IgG antibody response. Despite the number of reinfections, the highest IgM titer was 1:16 where the IgG response tended to increase with subsequent inoculations suggesting that the animals had developed an adaptive immune response. The focus of this group's work was studying the lower genital tract, but one case of acute endometritis and salpingitis was observed histologically upon autopsy. However, the authors did not have microbiologic studies of the upper tract prior to autopsy and were unable to identify intracytoplasmic chlamydial inclusions in epithelial cells.

Table 1: List of NHP used in reproductive health research.

Animal	Species	Average Life span (years)	Average size (cm)	Average weight (kg)	Menstrual length (days)*	Advantages/disadvantages
Olive baboon	*Papio anubis*	25–30	M: 70 F: 60	M: 24 F: 14.7	30–35 [23]	Advantages: anatomical and physiological closeness to humans, large size (facilitates procedures), straight cervix, readily available, non-threatened, can visually monitor cycle stage with greatest precision Disadvantages: large size and strength, large housing requirements
African Green Monkey (Vervet, Grivet) [24, 25]	*Chlorocebus aethiops* (formerly *Cercopithecus aethiops)*	11–13 (captive)	M: 49 F: 42.6	M: 5.5 F: 4.1	30–32 [26]	Advantages: anatomical and physiological closeness to humans, manageable size, readily available, non-threatened Disadvantages: tortuous Cervix, cannot visually monitor cycle stage
Common marmoset	*Callithrix jacchus*	12 (wild)	M: 18.8 F: 18.5	.350–.450* [27]	22–28	Advantages: anatomical and physiological closeness to humans, manageable size, readily available, non-threatened Disadvantages: no menstruation (has estrus cycle) [4], small size (precludes certain procedures); ovulation pattern slightly different from humans (twinning is the normal state), cannot visually monitor cycle stage
Macaques						
Pigtailed	*Macaca nemestrina*	26	M: 49.5–56.4 F: 46.7–56.4	M: 6.2–14.5 F: 4.7–10.9	30–40 [28]	Advantages: anatomy of reproductive tract tissues and physiology of menstrual cycle similar to humans, size (facilitates most procedures), non-threatened, can visually monitor cycle stage to some degree Disadvantages: limited availability, some species are seasonal breeders (rhesus, possibly Formosan rock macaque), tortuous cervix, aggressive temperament (rhesus), zoonotic disease risk
Formosan rock macaque (Taiwan monkey)	*Macaca cyclopis*	NA	40–55*	4–8*	24–31	
Rhesus	*Macaca mulatta*	25	M: 53.2 F: 46.9	M: 7.7 F: 5.34	28	
Cynomolgus	*Macaca fascicularis*	31	M: 41–65 F: 39–50	M: 4.7–8.3 F: 2.5–5.7	28–32 [29, 30]	

NA: not available. *Differences between males and females were not noted.

5. Development of PID in NHPs

Concomitant with Johnson et al.'s work in marmosets, Moller et al. did similar work in grivet monkeys (Table 2) [46]. By directly inoculating the fallopian tubes and the endometrial cavity, grivet monkeys developed acute self-limited salpingitis. The histological findings in the tubal tissues post-inoculation (infiltration of the subepithelial and mucosal epithelium, abundant lymphocytes, and polymorphonuclear (PMN) leukocytes, and exudate in the tubal lumen with small clusters of desquamated epithelium) mirrored what is considered to be the hallmark signs of acute salpingitis in women [32]. Moller also showed that there was an activated immune response associated with inoculation with *C. trachomatis*. The antibody response was that of a primary infection with an IgM to IgG antibody seroconversion [32]. Additionally, in the animal that was infected in the endometrial cavity they documented ascent of the *C. trachomatis* to the fallopian tubes.

A later study by this same group found that direct cervical inoculation with *C. trachomatis* resulted in acute salpingitis [12]. These findings confirmed Johnson's results in the marmoset. They, too, satisfied Koch's postulates and reisolated the organism. Again, the cervical infection with *C. trachomatis* resulted in "classic" histological findings for acute salpingitis as noted in humans, and like the previous trial, the serological conversion seen post cervical inoculation was an IgM to IgG seroconversion.

6. Current NHP Models of PID

Using direct tubal inoculation, Patton and colleagues demonstrated a histopathologically similar acute salpingitis in the pigtailed macaque to that noted by Moller et al. in the grivet monkey (Table 2) [36, 37]. In the pigtailed macaques and grivet monkeys a single inoculation of *C. trachomatis* resulted in self-limited tubal inflammation without evidence

Table 2: Historical NHP PID models and the pathological features found with each subsequent trial.

Researcher, Year	Animal	Chlamydial inoculum	Site of inoculation	Pathologic features
Thygeson and Mengert 1936 [11]	Baboon	Material from infant with inclusion blennorrhea	cervix	Cervicitis, purulent cervical discharge
Alexander et al., 1967 [31]	Taiwan monkey	TRIC	cervix	Mild erythema of the lower genital tract
Ripa et al., 1979 [32]	Grivet	Ct, 2×10^5 IFU/mL	fallopian tube	Histological changes in the upper genital tract, swollen reddened tubes, abundant lymphocytes in the tubal epithelium, clusters of desquamated cells, adhesions between mucosal folds
Johnson et al., 1980 [20]	Marmoset	Ct, 5×10^5 IFU/mL	vagina	Acute inflammatory reaction of the lower genital tract, PMNs, intracytoplasmic chlamydial inclusions
Moller et al., 1980 [33]	Grivet	Ct, 2×10^5 IFU/mL	cervix	Reddened and swollen tubes, exudate from ostia, histological changes in the upper genital tract, lumen of tube diminished and tubal epithelium atrophic and flattened, demonstrated vertical spread of organism
Johnson et al., 1981 [34]	Marmoset	Ct, 4.2–8.8×10^5 IFU/mL	vagina	Demonstrated that reinfection with either homologous or heterologous strain could result in infection however the shorter duration between inoculations resulted in marked immunity and decreased duration of infection
Johnson et al., 1985 [35]	Marmoset	Ct, 5×10^5 IFU/mL	vagina	Cervical erythema with occasional edema, cloudy, or purulent mucus, PMNs identified, endometritis, and salpingitis
Patton et al., 1983, 1984 [36, 37]	Pigtailed macaque	Ct, 6×10^6 IFU/mL	fallopian tube	Acute salpingitis with marked edema and swelling, flocculent exudate, PMNs, isolation of Ct from cervix and tubes
Patton et al., 1987 [38]	Cynomolgus rhesus	Ct, 7×10^6 IFU/mL	Subcutaneous pocket model with fallopian tube implants	Marked erythema, edema, and swelling, widespread inflammation with lymphocytic cells and PMNs, and plasma cells had infiltrated the stroma, infection duration shorter than that seen in intact model with direct tubal inoculation
Patton et al., 1987 [14]	Pigtailed macaque	Ct, $2–4 \times 10^8$ IFU/mL	fallopian tube	Chronic salpingitis with extensive tubal scarring, distal tubal obstruction, and peritubal adhesions
Patton et al., 1990 [39]	Pigtailed macaque	Ct, 1×10^6 IFU/mL	cervix	Acute and chronic salpingitis, peritubal adhesions
Wolner-Hanssen et al., 1991 [40]	Pigtailed macaque	Ct, 1×10^6 IFU/mL	cervix	Demonstrated that repeated cervical inoculation resulted protective immunity though there was no relationship between the antibody titer and reinfection
Patton et al., 1994 [41]	Pigtailed macaque	Ct, 5×10^3 IFU/mL	Subcutaneous pocket model with fallopian tube implants	Demonstrated a delayed hypersensitivity in response to inoculation with Ct in both previously infected pockets and noninfected pockets
Van Voorhis et al., 1997 [42]	Pigtailed macaque	Ct, 1×10^5 IFU/mL	Subcutaneous pocket model with fallopian tube implants, cervix and fallopian tubes	Suggests that a Th1-like cytokine response is seen with repeated infection with Ct
Patton et al., 2005 [43]	Pigtailed macaque	Ct, 1×10^5 IFU/mL	cervix	Demonstrated the azithromycin treatment in Ct infection ameliorated the immune response to Ct infection
Patton et al., 2008, 2009 [44, 45]	Pigtailed macaque	Ct, 5×10^5 IFU/mL	vaginal fornix, rectum	Demonstrated the ability of NHP PID model for evaluating the safety and efficacy of topical microbicides
Bell et al., 2010 [21]	Olive baboon	Ct, 1×10^7 IFU/mL	cervix	Acute and chronic salpingitis, peritubal adhesions

Ct: *Chlamydia trachomatis.*

of tubal scarring. This inflammatory period peaked at day 14 and resolved by days 28–35. However, with repeated inoculations a chronic salpingitis with extensive tubal scarring, distal tubal obstruction, and peritubal adhesions was induced [14].

This picture of chronic salpingitis after repeated infections led Patton and colleagues to develop a subcutaneous pocket model in cynomolgus, rhesus and pigtailed monkeys so that the tubal response to infection could be easily monitored [38]. The histopathology again showed a progression of the inflammation of the mucosal and muscular layers as well as a transition from lymphocytic cells, PMNs, and plasma cells. Like previous studies this model showed a short-lived infection that was microbiologically, immunologically, and histopathologically similar to studies in intact monkeys where inoculation had occurred directly to the fallopian tube [36]. An important advantage of the pocket model is that separate anatomic tubal sites are established and can be individually manipulated per study design. However, duration of infection was shorter. Though this model was successful in studying the kinetics of acute and chronic chlamydial infection in tubal tissues, the use of the intact NHP reproductive tract is essential to investigate (scarring and fibrosis) pathogenesis of *Chlamydia*-induced PID.

Recognizing that the intact NHP model provided a more analogous model of human disease Patton and colleagues investigated how infection ascends from the lower into the upper reproductive tract. Repeated weekly (×five weeks) cervical inoculations in four pig-tailed macaques caused peritubal adhesions in all four monkeys. In contrast, in a separate study, after primary cervical inoculation, seven animals were rechallenged at the cervix only after the cervix had become culturally negative for two consecutive weeks; none developed adhesions [39].

With this new model of upper tract infection Patton et al. had a system that could be used to evaluate cellular immunity associated with chlamydial infections and chronic immune responses to infection that are associated with the tubal damage. Additionally, in another study it was demonstrated with repeated inoculations (after cessation of infection) animals had a shorter duration of infection [40]. With this protective immune response there was no correlation between the antibody titers (IgG) at the time of reinoculation and the success of reinoculation. Attempting to further define the immune response associated with chlamydial infection Patton et al. used their subcutaneous pocket model to better understand this association [41]. They showed the same histological changes in the tubal tissues as seen in their previous work; however, after resolution with primary infection several pockets were tested by injection of rhsp60 (recombinant heat-shock protein 60) or sham injection. Those pockets that were injected with rhsp60 showed a marked increase in inflammation at 24 hours and an even greater response at 48 hours. After 48 hours the cellular infiltrate consisted primarily of mononuclear lymphocytes that permeated the submucosal tissues, which is consistent with a delayed hypersensitivity response [41]. Later, again using the subcutaneous pocket model as well as cervical/tubal inoculations mRNAs for IFN-γ, IL-2, IL-6, IL-10, but not IL-4, were isolated from salpingeal tissues [42]. IFN-γ and IL-2 are made by TH1 CD4 T cells, whereas TH2 cells make IL-4. IL-6 and IL-10 are made by both TH1 and TH2 cells. These results suggested that TH1-like cytokines are made by repeated infection with *C. trachomatis* [42].

Recently, Patton et al. have used their model of PID to evaluate different antibiotic treatments for chlamydial infections [43]. In this study different treatment options (doxycycline, azithromycin) were tested versus placebo (untreated infection). As expected, animals that were in the placebo arm developed PID. However, in the two treatment arms there were differences. Those animals treated with doxycycline developed inflammatory cell profiles similar to those of the untreated animals. In contrast the azithromycin-treated animals had significantly reduced levels of inflammatory infiltrates. Azithromycin treatment ameliorated the immune response and was highly effective in eradicating *C. trachomatis* from the lower and upper reproductive tract tissues [43].

As a vaccine for *C. trachomatis* has been not yet been developed, recent studies have focused on novel ways to prevent infection. Female-controlled products, such as microbicides, provide a critical opportunity to prevent sexually transmitted infections. In 1999, Patton et al. were awarded a Sexually transmitted Disease Prevention-Primate Contract designed to comparatively assess the safety of topical microbicide products to cervical and vaginal tissues after repeated exposure and to evaluate their efficacy in preventing cervical chlamydial infection. Over 28 products have been evaluated (gels, films, capsules, etc.) for efficacy, safety and 9 for prevention of *C. trachomatis* infection [44]. Four of the candidate products tested were associated with tissue abnormalities that included epithelial friability, abrasion, and disruption [44]. These results demonstrated early warnings of products with potentially deleterious cervicovaginal effects, which lead to reformulation of several products [44]. In the rectal safety and efficacy studies, 12 products have been evaluated for safety and one was additionally tested for efficacy against rectal chlamydial infection. Two products had an unacceptable safety profile, and no protection was observed in prevention of acquisition of rectal chlamydial infection [45].

Until recently, the baboon had not been used in PID models. It is probable that the size, strength, temperament, and space required to use these animals have limited their use. However, next to the great apes (chimpanzee, gorilla, orangutan), the baboon is the animal most similar to humans in reproductive anatomy, physiology, and biochemistry, including hormonal fluctuations and cycling [47]. Another benefit of the baboon is that they have a straight (rectilinear) cervical canal, not tortuous like the chimpanzee and macaque, which permits transcervical procedures (i.e., endometrial sampling, intrauterine contraception placement) in a manner that mirrors practice in women [8]. Given this similarity to humans, the baboon has been used in vaccine trials for *C. trachomatis* [48]. As mentioned above one of the earliest trials to use *C. trachomatis* in baboons was in the 1930s by Thygeson and Mengert, [11]. Those trials showed that the baboon could become infected with *C. trachomatis* and develop cervicitis. Furthermore, work by Digiacomo and colleagues showed that the genital tract

of male baboons could be infected and continue to shed chlamydial organisms for approximately 3 months after initial inoculation [49].

Based on the studies previously conducted in the baboon, as well as the Patton model of PID in the macaque, Bell et al. recently developed a new model of PID in baboons (Table 2) [21]. This work was conducted in wild-caught olive baboons, and *C. trachomatis*was used as the infectious agent. Bell et al. showed that the baboon was highly susceptible to *C. trachomatis*. Moreover, that with even a single inoculation, the organism ascended to the upper reproductive tract, as both Johnson and Moller had shown in their models [21]. Bacteria caused changes consistent with infection in the upper reproductive tract. Like women with *C. trachomatis* infections, the animals in this trial developed varying degrees of infection, from mild cervicitis to PID. Furthermore, Bell and colleagues demonstrated that multiple inoculations of *C. trachomatis* were able to drive the infection into the upper reproductive tract (unpublished data), and thus, like the work of Patton, have established a working model of PID.

7. Discussion

Each of the models of PID above offers investigators the ability to test scientific questions in animals that are anatomically, biologically, and physiologically similar to humans. Although there are a number of animal models, each model reflects some aspect(s) of human disease and appropriate models that should be utilized to study the desired outcome. Interestingly, inoculation with *C. trachomatis* in NHPs does not lead to infection in all animals, with some animals even failing to show cervicitis. This observation further supports the similarity to variable clinical picture that is seen in women, where some women remain asymptomatic. This point serves to further strengthen the similarity between the NHP model of PID and the human disease state. The basis for this heterogeneity requires further attention.

8. Conclusions

Research concerning PID has progressed greatly in the past century and continues to advance each year. The use of NHPs has allowed and will continue to allow for a greater understanding of the disease. Through the ethical and humane use of NHP models, the field of reproductive infectious disease has continued to improve our scientific understanding of the disease and has helped to improve treatment measures. The continued use of these models will allow for investigators to answer questions about human disease that cannot be easily studied in humans.

Acknowledgments

J. D. Bell is funded by the National Institutes of Health K12 HD065257. This work was also supported by National Institutes of Health Grant HL078727 (D.M.A.), and Contract no. HHSN266200700013C (ABD. Contract no. N01-AI-70013) and WaNPRC Grant RR00166 (D.L.P).

References

[1] Centers for Disease Control and Prevention, *Sexually Transmitted Disease Surveillance, 2007*, US Department of Health and Human Services, Atlanta, Ga, USA, 2008.

[2] D. E. Soper, "Pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 116, no. 2, pp. 419–428, 2010.

[3] *World Health Organization Global Strategy for the Prevention and Control of Sexually Transmitted Infections: 2006–2015*, 2007.

[4] W. C. Hill, "The use of primates in biomedical studies: a review of suitable species," *Annals of the New York Academy of Sciences*, vol. 162, no. 1, pp. 7–14, 1969.

[5] A. Keith, "The extent to which the posterior segments of the body have been transmuted and suppressed in the evolution of man and allied primates," *Journal of Anatomy and Physiology*, vol. 37, pp. 18–40, 1902.

[6] A. Keith, "The modes of origin of the carotid and subclavian arteries from the arch of the aorta in some of the higher primates," *Journal of Anatomy and Physiology*, vol. 29, pp. 453–458, 1895.

[7] T. M. D'Hooghe, C. M. Kyama, D. Chai et al., "Nonhuman primate models for translational research in endometriosis," *Reproductive Sciences*, vol. 16, no. 2, pp. 152–161, 2009.

[8] D. Chai, S. Cuneo, H. Falconer, J. M. Mwenda, and T. D'Hooghe, "Olive baboon (Papio anubis anubis) as a model for intrauterine research," *Journal of Medical Primatology*, vol. 36, no. 6, pp. 365–369, 2007.

[9] W. F. MacKenzie and H. W. Casey, "Animal model of human disease: endometriosis in rhesus monkeys," *The American Journal of Pathology*, vol. 80, no. 2, pp. 341–344, 1975.

[10] R. W. Te Linde and R. B. Scott, "Experimental endometriosis," *American Journal of Obstetrics and Gynecology*, vol. 60, no. 5, pp. 1147–1173, 1950.

[11] P. Thygeson and W. F. Mengert, "The virus on inclusion conjunctivitis," *Archives of Ophthalmology*, vol. 15, pp. 377–410, 1936.

[12] B. R. Moller and P. A. Mardh, "Experimental salpingitis in grivet monkeys by Chlamydia trachomatis. Modes of spread of infection to the fallopian tubes," *Acta Pathologica et Microbiologica Scandinavica*, vol. 88, no. 2, pp. 107–114, 1980.

[13] A. Johnson and D. Taylor-Robinson, "Animal model of human disease: chlamydial genital tract infections, experimental infection of the primate genital tract with chlamydia trachomatis," *American Journal of Pathology*, vol. 106, pp. 132–135, 1982.

[14] D. L. Patton, C. C. Kuo, S. P. Wang, and S. A. Halbert, "Distal tubal obstruction induced by repeated Chlamydia trachomatis salpingeal infections in pig-tailed macaques," *The Journal of Infectious Diseases*, vol. 155, no. 6, pp. 1292–1299, 1987.

[15] N. F. Jacobs Jr., E. S. Arum, and S. J. Kraus, "Experimental infection of the chimpanzee urethra and pharynx with Chlamydia trachomatis," *Sexually Transmitted Diseases*, vol. 5, no. 4, pp. 132–136, 1978.

[16] I. Miyairi, K. H. Ramsey, and D. L. Patton, "Duration of untreated Chlamydial genital infection and factors associated with clearance: review of animal studies," *The Journal of Infectious Diseases*, vol. 201, no. 2, pp. S96–S103, 2010.

[17] P. M. Furr and D. Taylor-Robinson, "Enhancement of experimental Mycoplasma pulmonis infection of the mouse genital tract by progesterone treatment," *Journal of Hygiene*, vol. 92, no. 2, pp. 139–144, 1984.

[18] A. L. Barron, H. J. White, and R. G. Rank, "A new animal model for the study of Chlamydia trachomatis genital

infections: infection of mice with the agent of mouse pneumonitis," *The Journal of Infectious Diseases*, vol. 143, no. 1, pp. 63–66, 1981.

[19] H. E. Carlsson, S. J. Schapiro, I. Farah, and J. Hau, "Use of primates in research: a global overview," *American Journal of Primatology*, vol. 63, no. 4, pp. 225–237, 2004.

[20] A. P. Johnson, C. M. Hetherington, and M. F. Osborn, "Experimental infection of the marmoset genital tract with Chlamydia trachomatis," *British Journal of Experimental Pathology*, vol. 61, no. 3, pp. 291–295, 1980.

[21] J. Bell, L. Harris, and I. Bergin, "The baboon (Papio anubis) as a novel model of Chlamydia trachomatis pelvic inflammatory disease (PID) for testing intrauterine contraception (IUC) oral presentation," in *Proceedings of the 37th Annual Meeting of the Infectious Diseases Society for Obstetrics & Gynecology*, Santa Fe, NM, USA, August 2010.

[22] D. L. Patton and R. G. Rank, "Animal models for the study of pelvic inflammatory disease," in *Advances in Host Defense Mechanisms: Sexually Transmitted Diseases*, T. C. Quinn, Ed., vol. 8, pp. 85–111, Raven Press, 1992.

[23] E. Honore and S. Tardif, "Reproductive biology of Baboons," in *The Baboon in Biomedical Research*, J. L. VandeBerg, S. Williams-Blangero, and S. Tardif, Eds., Springer, Chicago, III, USA, 2009.

[24] K. Cawthon Lang, Primate Fact Sheets: Vervet (*Chlorocebus*) Taxonomy, Morphology, and Ecology, 2011, http://pin.primate.wisc.edu/factsheets/entry/vervet.

[25] J. Kingdon and TM B, *Chlorocebus aethiops*, 2011, http://www.iucnredlist.org.

[26] J. Seier, "Vervet monkey breeding," in *The Laboratory Primate: Handbook of Experimental Animals*, S. Wolfe-Coote, Ed., Elsevier, Amsterdam, The Netherlands, 2005.

[27] S. Rensing and A.-K. Oerke, "Husbandry and management of New World Species: marmosets and tamarins," in *The Laboratory Primate: Handbook of Experimental Animals*, S. Wolfe-Coote, Ed., pp. 175–180, Elsevier, Amsterdam, The Netherlands, 2005.

[28] H. M. Kubisch, C. Gagliardi, V. M. Williams, E. P. Ribka, and M. S. Ratterree, "In vitro fertilization in the pigtailed macaque (*Macaca nemestrina*)," *Theriogenology*, vol. 66, no. 4, pp. 749–754, 2006.

[29] E. Van Esch, J. M. Cline, E. Buse, C. E. Wood, E. P. C. T. de Rijk, and G. F. Weinbauer, "Summary comparison of female reproductive system in human and the cynomolgus monkey (*Macaca fascicularis*)," *Toxicologic Pathology*, vol. 36, no. 7, supplement, pp. 171S–172S, 2008.

[30] G. Weinbauer, M. Niehoff et al., "Physiology and endocrinology of the ovarian cycle in macaques," *Toxicologic Pathology*, vol. 36, no. 7, supplement, pp. 7S–23S, 2008.

[31] E. R. Alexander and W. T. Chiang, "Infection of pregnant monkeys and their offspring with TRIC agents," *American Journal of Ophthalmology*, vol. 63, no. 5, pp. 1145–1153, 1967.

[32] K. T. Ripa, B. R. Moller, and P. A. Mardh, "Experimental acute salpingitis in grivet monkeys provoked by Chlamydia trachomatis," *Acta Pathologica et Microbiologica Scandinavica*, vol. 87, no. 1, pp. 65–70, 1979.

[33] B. R. Moller, E. A. Freundt, F. T. Black, and F. Melsen, "Experimental infection of the upper genital tract of female grivet monkeys with Mycoplasma fermentans," *Journal of Medical Microbiology*, vol. 13, no. 1, pp. 145–149, 1980.

[34] A. P. Johnson, M. F. Osborn, and B. J. Thomas, "Immunity to reinfection of the genital tract of marmosets with Chlamydia trachomatis," *British Journal of Experimental Pathology*, vol. 62, no. 6, pp. 606–613, 1981.

[35] A. P. Johnson, M. J. Hare, G. D. Wilbanks et al., "A colposcopic and histological study of experimental chlamydial cervicitis in marmosets," *British Journal of Experimental Pathology*, vol. 65, no. 1, pp. 59–65, 1984.

[36] D. L. Patton, S. A. Halbert, and C. C. Kuo, "Host response to primary Chlamydia trachomatis infection of the fallopian tube in pig-tailed monkeys," *Fertility and Sterility*, vol. 40, no. 6, pp. 829–840, 1983.

[37] D. L. Patton, "Immunopathology and histopathology of experimental chlamydial salpingitis," *Reviews of Infectious Diseases*, vol. 7, no. 6, pp. 746–753, 1985.

[38] D. L. Patton, C. C. Kuo, and S. P. Wang, "Chlamydial infection of subcutaneous fimbrial transplants in cynomolgus and rhesus monkeys," *The Journal of Infectious Diseases*, vol. 155, no. 2, pp. 229–235, 1987.

[39] D. L. Patton, P. Wolner-Hanssen, S. J. Cosgrove, and K. K. Holmes, "The effects of Chlamydia trachomatis on the female reproductive tract of the Macaca nemestrina after a single tubal challenge following repeated cervical inoculations," *Obstetrics and Gynecology*, vol. 76, no. 4, pp. 643–650, 1990.

[40] P. Wolner-Hanssen, D. L. Patton, and K. K. Holmes, "Protective immunity in pig-tailed macaques after cervical infection with Chlamydia trachomatis," *Sexually Transmitted Diseases*, vol. 18, no. 1, pp. 21–25, 1991.

[41] D. L. Patton, Y. T. C. Sweeney, and C. C. Kuo, "Demonstration of delayed hypersensitivity in Chlamydia trachomatis salpingitis in monkeys: a pathogenic mechanism of tubal damage," *The Journal of Infectious Diseases*, vol. 169, no. 3, pp. 680–683, 1994.

[42] W. C. Van Voorhis, L. K. Barrett, Y. T. C. Sweeney, C. C. Kuo, and D. L. Patton, "Repeated Chlamydia trachomatis infection of Macaca nemestrina fallopian tubes produces a Th1-like cytokine response associated with fibrosis and scarring," *Infection and Immunity*, vol. 65, no. 6, pp. 2175–2182, 1997.

[43] D. L. Patton, Y. T. Cosgrove Sweeney, and W. E. Stamm, "Significant reduction in inflammatory response in the macaque model of chlamydial pelvic inflammatory disease with azithromycin treatment," *The Journal of Infectious Diseases*, vol. 192, no. 1, pp. 129–135, 2005.

[44] D. L. Patton, Y. T. Cosgrove Sweeney, and K. J. Paul, "A summary of preclinical topical microbicide vaginal safety and chlamydial efficacy evaluations in a pigtailed macaque model," *Sexually Transmitted Diseases*, vol. 35, no. 10, pp. 889–897, 2008.

[45] D. L. Patton, Y. T. C. Sweeney, and K. J. Paul, "A summary of preclinical topical microbicide rectal safety and efficacy evaluations in a pigtailed macaque model," *Sexually Transmitted Diseases*, vol. 36, no. 6, pp. 350–356, 2009.

[46] B. R. Moller, E. A. Freundt, and P. A. Mardh, "Experimental pelvic inflammatory disease provoked by Chlamydia trachomatis and Mycoplasma hominis in grivet monkeys," *American Journal of Obstetrics and Gynecology*, vol. 138, no. 7, pp. 990–995, 1980.

[47] T. M. D'Hooghe, J. M. Mwenda, and J. A. Hill, "A critical review of the use and application of the baboon as a model for research in women's reproductive health," *Gynecologic and Obstetric Investigation*, vol. 57, pp. 1–60, 2004.

[48] P. J. Provost and J. H. Vickers, "Attempted immunization against trachoma infection in baboons," *American Journal of Veterinary Research*, vol. 33, no. 3, pp. 599–605, 1972.

[49] R. F. Digiacomo, J. L. Gale, S. P. Wang, and M. D. Kiviat, "Chlamydial infection of the male baboon urethra," *British Journal of Venereal Diseases*, vol. 51, no. 5, pp. 310–313, 1975.

Hindawi Publishing Corporation
Infectious Diseases in Obstetrics and Gynecology
Volume 2011, Article ID 753037, 6 pages
doi:10.1155/2011/753037

Review Article

A Practical Approach to the Diagnosis of Pelvic Inflammatory Disease

Oluwatosin Jaiyeoba and David E. Soper

Department of Obstetrics and Gynecology, Medical University of South Carolina, P.O. Box 250619, Charleston, SC 29425, USA

Correspondence should be addressed to David E. Soper, soperde@musc.edu

Received 13 May 2011; Accepted 30 May 2011

Academic Editor: Thomas Cherpes

The diagnosis of acute pelvic inflammatory disease (PID) is usually based on clinical criteria and can be challenging for even the most astute clinicians. Although diagnostic accuracy is advocated, antibiotic treatment should be instituted if there is a diagnosis of cervicitis or suspicion of acute PID. Currently, no single test or combination of diagnostic indicators have been found to reliably predict PID, and laparoscopy cannot be recommended as a first line tool for PID diagnosis. For this reason, the clinician is left with maintaining a high index of suspicion for the diagnosis as he/she evaluates the lower genital tract for inflammation and the pelvic organs for tenderness in women with genital tract symptoms and a risk for sexually transmitted infection. This approach should minimize treating women without PID with antibiotics and optimize the diagnosis in a practical and cost-effective way.

1. Introduction

Acute PID is associated with significant sequelae including tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. To ameliorate these adverse outcomes, an approach to its diagnosis must promote the ability to intervene with antimicrobial therapy early on the course of this ascending infection. It is less important to accurately determine where the patient may lie along the continuum of this ascending inflammatory process (cervicitis, endometritis, salpingitis, or peritonitis) and more important to empirically initiate an appropriate antibiotic regimen when the diagnosis is suspected.

There is a wide variation in the symptoms, some of which fail to imply a pelvic etiology, associated with acute PID (Table 1). They may range from subtle or mild to severe. This requires the clinician to maintain a high index of suspicion for the diagnosis of PID. Alternatively, the signs of PID are limited to an inflammatory exudate from the lower genital tract and pelvic organ tenderness. The value of recognizing the symptoms associated with acute PID is based on their ability to trigger the clinician's evaluation of the pelvis. If pelvic examination fails to reveal evidence of inflammation (if there is no leukorrhea), then the diagnosis of PID is much less likely and antibiotic treatment can be withheld while the remaining diagnostic workup defines the diagnosis. However, evidence of lower genital tract inflammation and any pelvic organ tenderness suggests the advisability of initiating antimicrobial therapy for a diagnosis of PID.

Laparoscopy can confirm the presence of acute salpingitis in a patient with a clinical diagnosis of PID. However, laparoscopy cannot be used to dictate which patients are candidates for antimicrobial therapy as women without acute salpingitis still require antimicrobial therapy for a clinical diagnosis of endometritis without salpingitis. Therefore, despite laparoscopy being the gold standard for the diagnosis of acute salpingitis, its routine use is neither feasible nor recommended.

The clinical diagnosis of PID is imprecise. Most studies confirm the positive predictive value (PPV) of a clinical diagnosis of PID for salpingitis of 65% when confirmed by laparoscopy. No single historical, physical, or laboratory finding is reliably diagnostic for acute PID. We are therefore left with the challenge of diagnosing PID in such a way as to minimize its associated sequelae while at the same time not over treating all women with pelvic pain or other genital tract symptoms with antimicrobials.

Table 1: Symptoms in women with clinically suspected pelvic inflammatory disease.

Abdominal pain
Abnormal discharge
Intermenstrual bleeding
Postcoital bleeding
Fever
Urinary frequency
Low back pain
Nausea/vomiting

Data from [5].

Table 2: Signs and tests to increase the specificity of a diagnosis of salpingitis.

An additional sign and abnormal laboratory tests increase the specificity of the diagnosis of PID:
(i) Oral temperature >101 F (>38.3°C)
(ii) Elevated C-reactive protein (CRP)
(iii) Laboratory documentation of cervical *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.
The most specific criteria for diagnosis of PID include:
(i) Endometrial biopsy with histologic evidence of endometritis
(ii) Transvaginal sonography or MRI showing thickened, fluid-filled tubes with or without free pelvic or tuboovarian complex or dopplers studies suggesting pelvic infection (tubal hyperemia)
(iii) Laparoscopic abnormalities consistent with PID

Data from [2].

2. Challenges

(1) Determine which women presenting with genital tract symptoms are candidates for antibiotic therapy for a diagnosis of acute PID.

(2) Determine which women with acute PID actually have acute salpingitis since these women are at the highest risk for the reproductive sequelae associated with this disease.

3. Composite Clinical Criteria

The diagnosis of PID should be considered in all sexually active women with or without lower abdominal pain and symptoms noted in Table 1. An assessment of risk for sexually transmitted infection (STI) enhances the specificity of these presenting symptoms. However, women without such risk factors should still have the diagnosis considered given that many will not be accurate in believing that they reside in a mutually monogamous sexual relationship [1]. Abdominal tenderness may not be present in many women with PID, particularly if peritonitis is not present or the patient has endometritis without salpingitis. A bimanual pelvic examination may reveal pelvic organ tenderness, uterine tenderness in the case of endometritis, and adnexal tenderness in the case of salpingitis. Cervical motion tenderness is another common finding in women with PID. The Centers for Disease Control and Prevention (CDC) [2] recommend empiric treatment for PID in sexually active young women (25 years old or younger) and other women at risk of STI (multiple sex partners or history of STI) if they are experiencing pelvic or lower abdominal pain, if no cause for the illness other than PID can be identified, and if one or more of the following is appreciated on bimanual pelvic examination: cervical motion tenderness, uterine tenderness, or adnexal tenderness. The limitation of this approach is that it fails to discriminate between the differential diagnoses of acute pelvic pain in reproductive-aged women. For this reason, the lower genital tract needs to be assessed for signs of inflammation. The cervical canal should be examined for the presence of yellow or green mucopus and friability. Microscopy of the vaginal secretions should be performed looking for leukorrhea (more than 1 leukocyte per epithelial cell). Evaluation for bacterial vaginosis (vaginal pH, clue cells, and whiff test) and trichomonas vaginitis is in order [3–6]. Finally, nucleic acid amplification testing (NAAT) for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* should be performed. If the cervix is normal and no white blood cells are noted during microscopy of the vaginal secretions, an alternative diagnosis should be investigated since this reliably excludes (negative predictive value 94.5%) upper genital tract infection [7]. Because the sensitivity of microscopy to detect *Trichomonas vaginalis* is relatively low (approximately 50%), symptomatic women with cervicitis and negative microscopy for trichomonads should receive further testing (i.e., culture or polymerase chain reaction method). Standardized diagnostic tests for *Mycoplasma genitalium* are not routinely performed. Empiric antibiotic treatment should be initiated in sexually active young women, especially those at risk for sexually transmitted infections (STIs), with pelvic or lower abdominal pain, if no other causes other than PID can be identified and if the following minimum criteria are present on pelvic examination:

(i) lower genital tract inflammation (cervicitis and/or leukorrhea (>1 leukocyte per epithelial cell on microscopy of the vaginal secretions)),

(ii) any pelvic organ tenderness (e.g., cervical motion tenderness, uterine tenderness, or adnexal tenderness).

The above approach is sufficient to assure that women with PID will be treated appropriately with antibiotics. At least a third of these women will not have acute salpingitis, but never the less are candidates for antibiotic therapy. Given that antibiotic regimens are identical for the treatment of women with acute salpingitis regardless of degree of severity, there is no utility in confirming the diagnosis laparoscopically.

If women with the clinical diagnosis of PID were to undergo routine laparoscopy, visual evidence of acute tubal inflammation (erythema, edema, and purulent exudate) would be confirmed approximately 65% of the time [8, 9]. Therefore, the clinical diagnosis of PID may represent women with visually confirmed acute salpingitis. However,

the clinical diagnosis of PID may also represent women with cervicitis and endometritis without salpingitis or with cervicitis alone [10, 11]. *C. trachomatis, N. gonorrhoeae*, bacterial vaginosis, and trichomonas vaginitis are associated with histologic evidence of endometritis in women without the clinical manifestations of PID [10]. The symptoms and signs of PID are essentially indistinguishable among women with acute salpingitis, those with endometritis without acute salpingitis, and those with cervicitis but neither endometritis nor salpingitis [11–13].

Other ancillary tests (Table 2) that can be useful in diagnosing PID include a complete blood count, erythrocyte sedimentation rate (ESR), or C-reactive protein (CRP). These tests are recommended for patients with clinically severe PID. Imaging studies are most helpful when ruling out competing differential diagnoses such as the use of pelvic ultrasonography to rule out symptomatic ovarian cysts and computed tomography to rule out appendicitis. Pelvic ultrasonography has limited sensitivity for the diagnosis of PID, but the specific finding of thickened fluid-filled tubes by ultrasonography supports the diagnosis of upper genital tract inflammation [14]. Pelvic ultrasonography should be ordered in patients requiring hospitalization or those with a pelvic mass.

4. Laboratory Tests

White blood cell (WBC) counts are beneficial when abnormal. However, only 60% of patients with PID present with elevated serum WBC count [15]. ESR, a nonspecific inflammatory marker has been found to be elevated in PID but an elevated ESR (>15 mm/h) is only present in approximately 75% of women with acute PID and, as a nonspecific maker of inflammation, can be found in other disease states. CRP, another inflammatory marker, has been studied in acute PID. In a series that involved 152 patients, a CRP >10 mg/dL had a good sensitivity (93%) and specificity (83%) in the diagnosis of PID [16]. Furthermore, CRP levels decrease to normal sooner than ESR following effective antibiotic therapy and may be beneficial as a monitoring tool.

There might be a role for CA-125 in PID diagnosis. Duk et al. from The Netherlands looked at the relationship of CA-125 in 50 patients with a provisional diagnosis of PID and concluded that the finding of an elevated serum CA-125 level confirms the diagnosis of peritoneal involvement in patients with a clinical diagnosis of PID [17]. They measured CA-125 concentrations in serum before laparoscopy and during hospitalization, using an enzyme immunoassay and found that CA-125 concentration before laparoscopy correlated with the extent of inflammatory peritoneal involvement and the predictive value of an elevated serum CA-125 level to indicate the presence of salpingitis (grades 1–3) was 97%. However, the predictive value of a normal CA-125 level indicating normal observations at laparoscopy (grade 0) was only 47%. Similarly, Mozas and coworkers [18] in Spain looked at the efficiency of different tumor markers (CA-125, carcinoembryonic antigen, CA-15.3, CA-19.9) and insulin-like growth factor I (IGF-I) measurements as a screening procedure for acute PID, and found no differences in the levels of CA-15.3, CA-19.9, carcinoembryonic antigen and IGF-I between three groups studied. However, the serum levels of CA-125 were significantly higher in patients who had PID and they concluded that measurement of serum CA-125 concentrations is recommended as a useful test for acute PID in patients undergoing laparoscopy for pelvic pain. Paavonen and coworkers in Finland measured serum levels of CA-125 in 31 patients with confirmed PID and found a correlation between CA-125 levels and the severity of adnexal inflammation as defined by laparoscopy. There was no association between isolation of specific microorganisms from the upper genital tract and elevated CA-125, and in most of the women in this study, serum levels of CA-125 decreased during treatment [19]. Finally, Moore and Soper [20] also reported a relationship between CA-125 and laparoscopically confirmed acute salpingitis and further noted that the degree of elevation of CA-125 levels correlated with severity of tubal inflammation.

5. Endometrial Biopsy

Endometrial biopsy has been studied extensively in the diagnosis of PID. It is less invasive compared with laparoscopy. The presence of neutrophils and plasma cells in the endometrium is indicative of endometritis and may be used to diagnose PID [21].

Kiviat and coworkers [22] looked at endometrial histopathology in 69 patients with clinically suspected acute PID and reported that 54% of the patients had both upper genital tract infection (UGTI) and laparoscopically confirmed salpingitis. They reported UGTI without salpingitis in 1%, while salpingitis without UGTI was reported in 16%. The study found that the simultaneous presence of five or more neutrophils per ×400 field in endometrial surface epithelium, together with one or more plasma cells per ×120 field in endometrial stroma were the best predictor of upper genital tract infection plus salpingitis. This combination had a sensitivity of 92% and a specificity of 87% for predicting the diagnosis of both UGTI and laparoscopically confirmed acute salpingitis. Additionally, 90% of all UGTIs identified in this study were attributable to *C. trachomatis* or *N. gonorrhoeae,* and 92% of the women diagnosed with UGTI and salpingitis had either chlamydia or gonorrhea infection.

6. Imaging Studies

Ultrasonography is the imaging method of choice, followed closely by Magnetic Resonance Imaging (MRI). Computed tomography (CT) is reserved for evaluation of the extent of PID within the abdomen and interventional management. Ultrasonography is noninvasive, widely available, and a good diagnostic tool to have in a physician's armamentarium for PID diagnosis. The typical ultrasound findings in acute PID have been described by Timor-Tritsch and Rottem [14], and the addition of Power Doppler to transvaginal ultrasonography has been found to increase its sensitive in diagnosis of PID. Transvaginal ultrasonography is preferred to transabdominal approach and also helpful in guiding needles to drain abscesses. MRI is expensive but more sensitive.

Tukeva et al. [23] compared transvaginal ultrasonography, MRI, and laparoscopy in 30 in-patients hospitalized in Finland with clinically suspected PID and reported that MRI diagnoses were 95% correct in 21 women with laparoscopically acute salpingitis compared with transvaginal sonogram that was 81% accurate. The sensitivity of MRI in the diagnosis of PID was found to be 95%, with a specificity of 89%, and overall accuracy was 93%. For transvaginal US, the corresponding value was 81%, 78%, and 80%, respectively. MRI is more accurate than transvaginal US and provides information about the differential diagnosis of PID, and as such its use may also reduce the need for diagnostic laparoscopy.

Although the literature is replete with reports regarding the sonographic findings of PID, little was published about CT images until the last decade [24]. CT findings in early PID include obscuration of the normal pelvic floor fascial planes, thickening of the uterosacral ligaments, cervicitis, oophoritis, salpingitis, and accumulation of simple fluid in the endometrial canal, fallopian tubes, and pelvis. The simple fluid may become complex as the disease progresses and eventually become a frank tuboovarian or pelvic abscess. Reactive inflammation can manifest as small or large bowel ileus or obstruction, hydronephrosis or hydroureter, and right upper quadrant inflammation (Fitz-Hugh-Curtis syndrome). One drawback of CT images however is the exposure to ionizing radiation, which can be problematic in young women.

If imaging is considered, we would first recommend transvaginal ultrasound, and if classic findings of PID are noted on ultrasound [14], no further imaging is required. If additional characterization is warranted, then we recommend MRI over CT because its overall accuracy is greater than 93% and does not carry the additional risk of ionizing radiation. If tuboovarian abscess (TOA) is suspected, we recommend an initial transvaginal ultrasound because this is the most cost effective imaging to allow percutaneous drain placement. However, many interventional radiologists will prefer CT to guide drain placement.

7. Laparoscopy

Laparoscopy has been shown to add considerable accuracy to the clinical methods of diagnosing acute salpingitis [9]. The procedure does not aggravate the inflammatory process. Jacobson and Weström looked at 905 cases over an eight-year period (1960–1967) and set the standard for laparoscopic diagnosis. The minimum laparoscopic criteria for visual diagnosis of acute salpingitis include: pronounced hyperemia of the tubal surface, edema of the tubal wall, and, thirdly, a sticky exudate on the tubal surface and from the fimbriated ends when patent. In their study, they hardly encountered difficulties differentiating mild pathologic changes and normal conditions, but one major drawback that can be envisaged is the patient with endometritis who has no salpingitis. In 814 cases in their series with suspected acute PID, 532 (65%) had laparoscopically confirmed acute salpingitis, 12% had other pathologic conditions, and 23% had no pathologic conditions changes. We suspect that a significant proportion of women in the latter category had endometritis without salpingitis.

In another study comparing clinical and laboratory findings with laparoscopic findings of acute PID, Eschenbach and coworkers [25] reported that the severity of clinical and laboratory manifestations (other than adnexal mass) was not associated positively with tubal occlusion and that the severity of some findings was actually associated negatively with the severity of tubal damage.

Although laparoscopy is referred to as the "gold standard" for the diagnosis of PID, review of the literature regarding its accuracy has been mixed. Accuracy of a clinical diagnosis when compared with diagnostic laparoscopy in the diagnosis of PID has been reported in various studies. Morcos et al. [26] in a study of 176 women with clinically diagnosed PID established laparoscopically confirmed PID in 76.1% of the cohort. Similarly, Cohen et al. [21] in a prospective case-control study investigated the etiology of acute salpingitis in a cohort of Kenyan women and confirmed salpingitis laparoscopically in 142 (90%) of the 158 women with a clinical diagnosis of acute PID. Conversely, Peipert et al. [27] found the sensitivities of both the accepted clinical criteria and the triad of laparoscopy visualization of edema, erythema and purulent exudates to be low. In that study, the sensitivities of the CDC's minimal clinical criteria for PID and the laparoscopic triad of edema, erythema, and purulent exudates were 65% and 60%, respectively. Sellors et al. [28] also found laparoscopy to be 50% and 80% sensitive and specific, respectively. In this study, additional evidence from endometrial and fimbrial biopsy increased the prevalence of confirmed PID from 30% in visual diagnosis alone to 46% when endometrial and fimbrial minibiopsy evidence was included.

Women with a recurrent diagnosis of PID and persistently negative NAATs and who are classified as "lower risk" epidemiologically should have laparoscopy to consider alternative diagnoses such as endometriosis.

8. Conclusion

Diagnostic laparoscopy with concomitant endometrial biopsy (subsequently examined histologically) in women with cervicitis will accurately define the continuum of inflammation associated with a clinical diagnosis of PID. This approach will allow the clinician/investigator to define as to whether the patient/subject has cervicitis/endometritis/acute salpingitis, cervicitis/endometritis, or cervicitis alone. This comprehensive approach is neither practical nor cost effective for those not in a research setting.

A purely clinical approach using the findings of lower genital tract inflammation (leukorrhea) associated with pelvic organ tenderness will identify the vast majority of women with PID, and all are candidates for antibiotic therapy (Figure 1). We recommend this approach as the most practical and cost effective.

Finally, additional testing and imaging is important in two scenarios first, in differentiating alternative diagnoses such as ovarian cysts and appendicitis. Second, in the more seriously ill patient who needs additional evaluation to assess

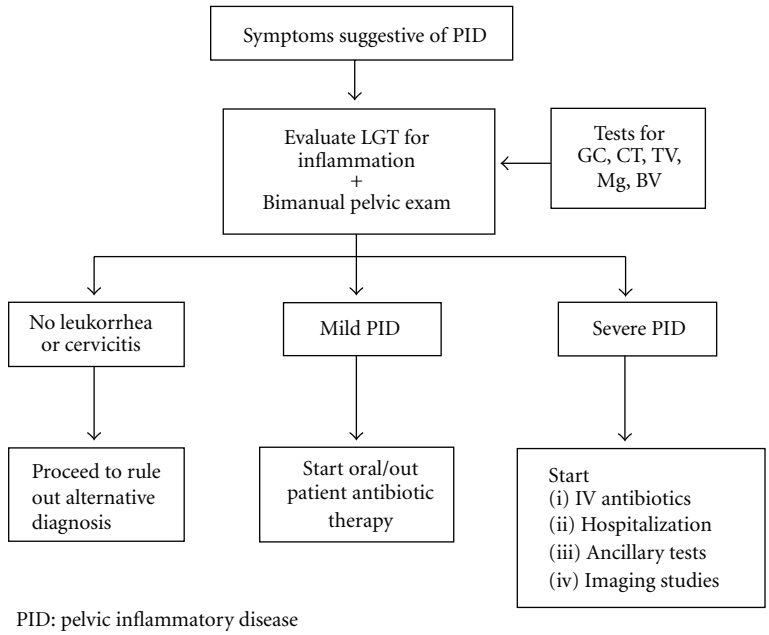


Figure 1: Flow chart Showing Clinical Diagnosis of PID.

the degree of sepsis and to consider the presence of a tuboovarian abscess. Women with severe PID are candidates for hospital admission and parenteral antibiotic therapy.

References

[1] A. M. Johnson, J. Wadsworth, K. Wellings, and J. Field, *Sexual Attitudes and Lifestyles*, Blackwell Scientific Publications, Oxford, UK, 1994.

[2] Centers for Disease Control and Prevention, "Sexually transmitted diseases treatment guidelines," *Morbidity and Mortality Weekly Report*, vol. 59, no. RR-12, 2010.

[3] D. E. Soper, N. J. Brockwell, H. P. Dalton, and D. Johnson, "Observations concerning the microbial etiology of acute salpingitis," *American Journal of Obstetrics and Gynecology*, vol. 170, no. 4, pp. 1008–1017, 1994.

[4] R. L. Sweet, "Role of bacterial vaginosis in pelvic inflammatory disease," *Clinical Infectious Diseases*, vol. 20, no. 2, pp. S271–S275, 1995.

[5] D. A. Eschenbach, T. M. Buchanan, H. M. Pollock et al., "Polymicrobial etiology of acute pelvic inflammatory disease," *New England Journal of Medicine*, vol. 293, no. 4, pp. 166–171, 1975.

[6] J. Paavonen, C. W. Critchlow, T. DeRouen et al., "Etiology of cervical inflammation," *American Journal of Obstetrics & Gynecology*, vol. 154, pp. 556–564, 1986.

[7] M. H. Yudin, S. L. Hillier, H. C. Wiesenfeld, M. A. Krohn, A. J. Amortegui, and R. L. Sweet, "Vaginal polymorphonuclear leukocytes and bacterial vaginosis as markers for histologic endometritis among women without symptoms of pelvic inflammatory disease," *American Journal of Obstetrics and Gynecology*, vol. 188, no. 2, pp. 318–323, 2003.

[8] I. Simms, F. Warburton, and L. Weström, "Diagnosis of pelvic inflammatory disease: time for a rethink," *Sexually Transmitted Infections*, vol. 79, no. 6, pp. 491–494, 2003.

[9] L. Jacobson and L. Weström, "Objectivized diagnosis of acute pelvic inflammatory disease," *American Journal of Obstetrics and Gynecology*, vol. 105, no. 7, pp. 1088–1098, 1969.

[10] H. C. Wiesenfeld, S. L. Hillier, M. A. Krohn et al., "Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease," *Obstetrics and Gynecology*, vol. 100, no. 3, pp. 456–463, 2002.

[11] L. O. Eckert, S. E. Hawes, P. K. Wölner-Hanssen et al., "Endometritis: the clinical-pathologic syndrome," *American Journal of Obstetrics and Gynecology*, vol. 186, no. 4, pp. 690–695, 2002.

[12] H. C. Wiesenfeld, R. L. Sweet, R. B. Ness, M. A. Krohn, A. J. Amortegui, and S. L. Hillier, "Comparison of acute and subclinical pelvic inflammatory disease," *Sexually Transmitted Diseases*, vol. 32, no. 7, pp. 400–405, 2005.

[13] P. Wolner Hanssen, P. A. Mardh, L. Svensson, and L. Westrom, "Laparoscopy in women with chlamydial infection and pelvic pain: a comparison of patients with and without salpingitis," *Obstetrics and Gynecology*, vol. 61, no. 3, pp. 299–303, 1983.

[14] I. E. Timor-Tritsch and S. Rottem, "Transvaginal ultrasonograpic study of the fallopian tube," *Obstetrics and Gynecology*, vol. 70, pp. 424–428, 1987.

[15] D. E. Soper, "Surgical considerations in the diagnosis and treatment of pelvic inflammatory disease," *Surgical Clinics of North America*, vol. 71, no. 5, pp. 947–962, 1991.

[16] M. Hemila, L. Henriksson, and O. Ylikorkala, "Serum CRP in the diagnosis and treatment of pelvic inflammatory disease,"

Archives of Gynecology and Obstetrics, vol. 241, no. 3, pp. 177–182, 1987.

[17] J. M. Duk, F. M. Kauer, G. J. Fleuren, and H. W. A. De Bruijn, "Serum CA 125 levels in patients with a provisional diagnosis of pelvic inflammatory disease. Clinical and theoretical implications," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 68, no. 7, pp. 637–641, 1989.

[18] J. Mozas, J. A. Castilla, P. Jimena, T. Gil, M. Acebal, and A. J. Herruzo, "Serum CA-125 in the diagnosis of acute pelvic inflammatory disease," *International Journal of Gynecology and Obstetrics*, vol. 44, no. 1, pp. 53–57, 1994.

[19] J. Paavonen, A. Miettinen, P. K. Heinonen et al., "Serum CA 125 in acute pelvic inflammatory disease," *British Journal of Obstetrics and Gynaecology*, vol. 96, no. 5, pp. 574–579, 1989.

[20] E. Moore and D. E. Soper, "Clinical utility of CA125 levels in predicting laparoscopically confirmed salpingitis in patients with clinically diagnosed pelvic inflammatory disease," *Infectious Diseases in Obstetrics and Gynecology*, vol. 6, no. 4, pp. 182–185, 1998.

[21] C. R. Cohen, N. R. Mugo, S. G. Astete et al., "Detection of mycoplasma genitalium in women with laparoscopically diagnosed acute salpingitis," *Sexually Transmitted Infections*, vol. 81, no. 6, pp. 463–466, 2005.

[22] N. B. Kiviat, P. Wolner-Hanssen, D. A. Eschenbach et al., "Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis," *American Journal of Surgical Pathology*, vol. 14, no. 2, pp. 167–175, 1990.

[23] T. A. Tukeva, H. J. Aronen, P. T. Karjalainen, P. Molander, T. Paavonen, and J. Paavonen, "MR imaging in pelvic inflammatory disease: comparison with laparoscopy and US," *Radiology*, vol. 210, no. 1, pp. 209–216, 1999.

[24] J. W. Sam, J. E. Jacobs, and B. A. Birnbaum, "Spectrum of CT findings in acute pyogenic pelvic inflammatory disease," *Radiographics*, vol. 22, no. 6, pp. 1327–1334, 2002.

[25] D. A. Eschenbach, P. Wölner-Hanssen, S. E. Hawes, A. Pavletic, J. Paavonen, and K. K. Holmes, "Acute pelvic inflammatory disease: associations of clinical and laboratory findings with laparoscopic findings," *Obstetrics and Gynecology*, vol. 89, no. 2, pp. 184–192, 1997.

[26] R. Morcos, N. Frost, M. Hnat, A. Petrunak, and G. Caldito, "Laparoscopic versus clinical diagnosis of acute pelvic inflammatory disease," *Journal of Reproductive Medicine*, vol. 38, no. 1, pp. 53–56, 1993.

[27] J. F. Peipert, L. A. Boardman, and C. J. Sung, "Performance of clinical and laparoscopic criteria for the diagnosis of upper genital tract infection," *Infectious Diseases in Obstetrics and Gynecology*, vol. 5, no. 4, pp. 291–296, 1997.

[28] J. W. Sellors, J. Mahony, C. Goldsmith et al., "The accuracy of clinical findings and laparoscopy in pelvic inflammatory disease," *American Journal of Obstetrics and Gynecology*, vol. 164, no. 1 I, pp. 113–120, 1991.