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PELVIC INFLAMMATORY DISEASE SUPPLEMENT

A Review of the Challenges and Complexities in the Diagnosis, Etiology, Epidemiology, and Pathogenesis of Pelvic Inflammatory Disease

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Pelvic inflammatory disease (PID) is a syndrome that causes substantial morbidity, including chronic pelvic pain, to women globally. While limited data are available from low- and middle-income countries, national databases from the United States and Europe suggest that PID incidence may be decreasing but the rate of decrease may differ by the etiologic cause. Recent studies of women with PID have reported that fewer than half of women receiving a diagnosis of PID have gonococcal or chlamydial infection, while *Mycoplasma genitalium*, respiratory pathogens, and the constellation of bacteria associated with bacterial vaginosis may account for a substantial fraction of PID cases. The clinical diagnosis of PID is nonspecific, creating an urgent need to develop noninvasive tests to diagnose PID. Advances in serologic testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* could advance epidemiologic studies, while the development of vaccines against these sexually transmitted pathogens could affect incident PID and associated morbidity.

Keywords. PID; Chlamydia; gonorrhea; bacterial vaginosis; endometritis; *Mycoplasma genitalium*.

Pelvic inflammatory disease (PID) is a syndrome that predominately affects cisgender women of reproductive age and can lead to chronic pelvic pain and infertility. Endometritis, salpingitis, tubo-ovarian abscess, and pelvic peritonitis are all on the spectrum of inflammatory processes comprising PID. Because PID can be symptomatic or asymptomatic, it is often left undiagnosed and untreated. Even when symptoms occur, the diagnosis of PID based on clinical signs and symptoms is often inaccurate. Many women given a diagnosis of PID have no evidence of genital tract infection, while other women learn that they have had PID only when they learn that they have tubal factor infertility. Diagnosis of PID is complicated because it (1) can be a sequelae of sexually transmitted pathogens, including *Neisseria gonorrhoeae* or *Chlamydia trachomatis,* (2) can occur when respiratory or enteric pathogens infect the female genital tract, or (3) can result when bacteria and mycoplasmas that are part of the vaginal microbiome ascend into the upper genital tract and cause inflammation.

It is estimated that PID accounts for 94% of morbidity in women associated with sexually transmitted infections (STIs), including HIV in high-income countries [1]. Surprisingly, the burden of PID among women, measured in terms of disability adjusted life-years, was also higher than the burden of disease associated with HIV among men in an analysis by the World Bank [1]. Given the substantial burden of death and disease associated with HIV in men this may seem shocking [2]. Even though PID is rarely life-threatening, the long-term morbid effects are substantial. Thus, PID is responsible for a considerable disease burden and represents an important healthcare issue in industrialized countries [2]. Even though little data is available on PID in low- and middle-income countries, chronic pelvic pain and infertility undoubtedly represent a substantial global health burden. Scarring of the fallopian tube can also lead to ectopic pregnancy which is a cause of death in women of reproductive age globally.

Despite the prevalence and burden of PID in women, there has been limited progress in identifying new strategies to prevent the incidence and sequelae of PID. This objective of the current review is to highlight the epidemiology of PID, to explore the challenges with its diagnosis, to review the emerging data on the evolving etiology of this syndrome, and to highlight gaps in research on PID.

EPIDEMIOLOGY: TRENDS AND GAPS IN OUR KNOWLEDGE

For much of the world, there are limited contemporary data on the prevalence, incidence, or burden of PID. In the 1980s and 1990s there was a substantial body of research on the etiology, diagnosis, and treatment of PID in the United States and

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Europe [3, 4], but the relative investment in research directly related to better diagnosing and treating PID has been limited over the past 2 decades. The National Health and Nutrition Examination Survey 2013–2014, conducted in the United States, has provided estimates of PID in sexually experienced women aged 18–44 years [5] and reported that approximately 4.4% of all sexually experienced women and 10% of women with a previously diagnosed STI received a diagnosis of PID in their lifetime.

A follow-up study combined national data from the National Health and Nutrition Examination Survey and the National Survey of Family Growth, 2 nationally representative emergency department data sets, 2 nationally representative physician office data sets, and 2 sentinel surveillance sources for sexual health visits to assess the burden of PID in the United States among women aged 15–44 years between 2006–2016 [6]. Three of the 4 representative national samples showed overall declines in self-reported PID history, with small increases on PID in 2015. Based on these data, the authors estimated that 2 million reproductive age American women have had PID. There were differences in PID burden by age, race, and region, with the highest burden among black, non-Hispanic women and those living in the South [6]. Although declines in PID have also been reported among American Indian and Alaska Native women, PID remains a substantial health burden for ethnic/racial minorities in the United States [7].

These findings mirror the trends found in England. In a national data set from 2009 to 2019, there was a decline in the diagnosis of PID over the decade, with chlamydial PID declining by 58%, gonococcal PID by 34% and "nonspecific" PID by 37% [8]. The authors concluded that widespread chlamydial screening likely contributed to the substantial decline in chlamydial PID and may have contributed to a decline in nonchlamydial PID. Their hypothesis is that since chlamydial infection of the fallopian tubes leads to a persistent epithelial-to-mesenchymal transition state, it could increase susceptibility to infection by bacterial vaginosis (BV)–associated pathogens [9]. Thus, treatment of chlamydial infections could lead to reductions in both chlamydial and nonchlamydial PID.

Contemporary and high-quality epidemiologic studies are needed to identify whether public health measures like screening and treatment of STIs are affecting the prevalence of PID and to identify how much diagnosed PID is not associated with gonococcal or chlamydial infections. It will also be critically important in future epidemiologic studies to have better documentation of the etiology of PID in order to develop better diagnostic tools to identify the fraction of PID cases attributable to non-STI pathogens. Furthermore, data are needed to identify the sources of racial disparities in the diagnosis of PID and to develop interventions to address these disparities.

CHANGING ETIOLOGY OF PID

The reported etiology of PID has changed over the past 70 years as availability of accurate diagnostic testing and pathogen prevalence have changed. As summarized by Mitchell et al [10], in the 1950s PID was associated with *Mycobacterium tuberculosis* and *N. gonorrhoeae,* while in the 1980s most cases were attributed to gonococcal infection, though sensitive diagnostic tests for *C. trachomatis* were not yet available. The proportion of women with diagnosed PID who have gonococcal or chlamydial infection varies widely, depending on whether the sampling site is the cervix, the endometrium or the fallopian tubes, or the peritoneum, and whether detection was based on culture or nucleic acid amplification tests (NAATs). However, more than half of women with clinical signs and symptoms of PID who have histologically confirmed PID do not have either STI pathogen, even when sensitive NAATs are used [10]. A recent clinical trial of US women having symptomatic PID reported that only 25% had either STI [11]. Nonetheless, information posted for patients continues to advise women that PID is predominately due to gonococcal and/or chlamydial infection [12, 13].

What are the other etiologic causes of PID? While there is still uncertainty about the relative fraction of PID caused by other pathogens, facultative and anaerobic bacteria associated with BV-associated bacteria, *Mycoplasma genitalium,* and enteric pathogens can contribute to PID. There is evidence that anerobic and facultative bacteria from the vagina, especially BV-associated pathogens, can ascend to the endometrium and fallopian tubes to cause PID, something that was reported 4 decades ago [14]. Women with BV are more likely to have histologic evidence of endometritis [15, 16] and are to have anaerobic gram-negative rods and cocci detected by cultivation from the endometrium.

The inclusion of metronidazole, an antimicrobic agent with activity against BV, to treat women with acute PID has been demonstrated to reduce the prevalence of BV-associated pathogens, notably *Atopobium vaginae*, anaerobic gram-negative rods, and gram-positive cocci, in the endometrium a month after treatment [11]. Although there are more limited data on the presence of these pathogens in the fallopian tubes of women with PID, one study conducted in Kenya used 16S sequencing to identify pathogens in fallopian tube samples collected during surgery [17]. Of 45 women with acute salpingitis 25% had identified bacterial 16S sequences, including *A. vaginae* and *Leptotrichia* spp., compared with none of the 44 control women having tubal ligations [17].

BV-associated bacteria have also been linked with incident PID in longitudinal studies. Diagnosis of BV has been linked with an increased risk for incident PID in nearly 3000 women [18]. In a second longitudinal study, BV-associated bacteria in the vagina were also associated with a doubled risk of incident

PID [19]. Subsequent testing using quantitative polymerase chain reaction (PCR) in a case control study demonstrated that symptomatic PID was more likely to develop in women with *A. vaginae*, *Sneathia*, BVAB-TM7, *Megasphaera*, *Eggerthella*-like bacterium, *Mobiluncus*, *G. vaginalis*, BVAB1, BVAB2, *Mageeibacillus indolicus*, *Prevotella timonensis*, and *Prevotella amnii* in the vagina at higher densities [20].

Cervical *M. genitalium* has been linked to 2-fold increased risk of prevalent and incident PID [21] and the relative prevalence of *M. genitalium* is similar to that of *C. trachomatis* among women with acute PID [11], but the independent role of this pathogen in PID remains unclear. Although *M. genitalium* has been demonstrated in animal models to ascend to the upper genital tract and cause disease [22], in the randomized treatment trial of PID during which repeated endometrial biopsies were obtained after treatment, *M. genitalium* was less frequent at follow-up among women who received metronidazole even though this antimicrobial agent has no activity against mycoplasmas [11]. Just as Horner et al [9] have suggested that previous chlamydial infection may enhance upper genital tract infections by BV-associated bacteria, this treatment trial suggests that the presence of BV-associated microbiota may provide a local environment more conducive to infection by *M. genitalium*. More research on the role of *M. genitalium* in PID is needed to clarify these complex relationships.

Interestingly, respiratory and enteric pathogens have also been recovered from women having PID. While respiratory pathogens are not found at either high prevalence or abundance in the vaginal microbiome of adult women, these pathogens can cause vaginal infections [23] and can plausibly be transmitted from the oropharynx or rectum to the vagina during sex. Wiesenfeld et al [11] reported that *Hemophilus influenzae* was detected in the endometrial biopsy culture in 6 of 233 women (2%), while *N. gonorrhoeae* was detected by NAAT in 12 women (4%) with clinically diagnosed PID. *Streptococcus pyogenes* has also been reported to cause salpingitis in some reports [24].

DIAGNOSIS OF PID: CLINICAL DIAGNOSIS, PATHOGEN TESTING, SEROLOGICAL TESTS, AND PEPTIDE ARRAYS

Criteria recommended by the Centers for Disease Control and Prevention for the diagnosis of PID include uterine, adnexal, and/or cervical motion tenderness [25], which are recognized to have a moderate to high sensitivity but low specificity for acute PID diagnosis [26]. This approach, which results in overtreatment of women who do not have evidence of upper genital tract inflammation, has been deliberate to ensure the fewest number of women who have PID be left untreated. In the PEACH study of >800 women with mild or acute PID, many had no evidence of upper genital tract infection or inflammation [27]. Testing that has been included in research studies, including endometrial biopsies to collect tissue samples for STI and other testing as well as histology, is invasive and not implementable in primary care and emergency medicine settings. However, it is plausible that the specificity of the clinical PID diagnosis could be enhanced by incorporating results of laboratory tests. As described by Soper and Wiesenfeld [26] blood-based tests, including erythrocyte sedimentation rate, C-reactive protein, and CA-125, have all been evaluated as diagnostic indicators of upper genital tract inflammation, but none were demonstrated to improve diagnosis compared with history and physical examination.

The lack of specificity in the clinical diagnosis of PID has led to efforts to identify noninvasive PID biomarkers to identify the subset of women having upper genital tract inflammation. One such approach, by Hillier et al [28], proposed the use of a panel comprised of quantitative PCR tests from a lower genital tract swab sample for *C. trachomatis, Gardnerella vaginalis, A. vaginae, P. amnii,* and *Lactobacillus crispatus.* The authors evaluated the utility of this approach in 31 women having acute endometritis and 138 women without histologic endometritis and found that a score based on the presence of *C. trachomatis* and/or high concentrations of BV-associated bacteria and the absence of *L. crispatus* was 87% sensitive and 74% specific at identifying the subset of women having acute inflammation in the endometrium [28]. While limited by its relatively small size and its being conducted in only 1 clinical site, this study suggests that multiplex PCR for specific lower genital tract microorganisms may be used to identify the subset of women having endometritis.

Another potential area of research is related to the use of blood microarray analyses as pathogen-specific biomarkers of PID. Zheng et al [29] compared blood messenger RNA responses in 14 women with chlamydial and/or gonococcal PID and histologically confirmed endometritis with responses in 16 women with infections limited to the cervix. Twelve asymptomatic women with no STIs or upper tract infection were included as controls. Unsupervised hierarchical clustering using 4952 gene transcripts revealed that women with gonococcal or chlamydial PID had overexpression of myeloid cell genes and suppression of protein synthesis, mitochondrial oxidative phosphorylation, and T-cell–specific genes.

IMMUNOPATHOGENESIS OF PID

The lack of high-quality assays for the accurate detection of past infection due to *C. trachomatis* has been a barrier to gaining a full understanding of the role of past chlamydial infection in PID. Because *C. trachomatis* and *C. pneumoniae* infections are so common in humans, and there is cross-reactivity across chlamydial species, assays based on detection of chlamydial elementary bodies and or outer membrane antigens have poor specificity [29]. Efforts to develop species-specific assays based on peptide antigens for molecular serology of chlamydia [29] have yielded some success in bringing a higher level of specificity

to these assays compared with commercial assays [30, 31]. The development of a microarray platform for the peptide antigen assay could enable high-throughput screening of candidate peptides for serologic fingerprinting of chlamydial infection, including those associated with PID [32]. Serum samples from well-characterized patients with well-defined lower and/or genital tract infections will be essential to exploiting the power of these assays to clarify the role of chlamydial infections in PID.

Unlike with *C. trachomatis,* for which serologic work has been used to estimate past infection, it has been difficult to identify serologic tests for *N. gonorrhoeae* exposure that accurately identify past exposure to this pathogen, or that can predict immunity or the likelihood of developing PID [33]. *N. gonorrhoeae* has broad antigenic variability in the lipooligosaccharide, the porin protein (Por B), the type 4 pilus, Opa proteins, and transferrin-binding proteins A and B [33]. In addition, horizontal gene transfer occurs between gonococci and other *Neisseria* species, allowing for homologous recombination between antigen segments. Therefore, the development of a standard antigen preparation for use in serologic and cellular assays to measure immunity has been problematic. There is some evidence that past gonococcal salpingitis provides some evidence of reinfection [34], and that highly exposed sex workers in Kenya had evidence of porin serovar specific immunity [35]. Nonetheless, there is no suitable serologic test available to predictably identify women who have had gonococcal infection of the lower or upper genital tract disease due to this pathogen.

N. gonorrhoeae manipulates host immune responses for its own survival [33]. For example, gonococcal Opa proteins bind to T lymphocytes, resulting in their inactivation. Studies in the mouse model have demonstrated that gonococcal infection induces innate immune responses while suppressing adaptive immune responses by increasing the production of regulatory cytokines and regulatory T lymphocytes [36, 37]. A gap in our understanding is how gonococcal infection affects immune responses, both adaptive and innate, and studies designed to catalog cytokine responses and how they affect immune induction and regulation are critically needed [33].

STRATEGIES AND OPPORTUNITIES TO AFFECT PID

Prevention of PID could be greatly affected by the availability of effective vaccines against *N. gonorrhoeae* and *C. trachomatis*. There have been significant advances in the development of models that can be used for preclinical testing of vaccines against gonococcal PID [38], and the mouse model has provided a useful tool to directly evaluate leads from epidemiologic data. For example, a New Zealand study of the meningococcal outer membrane vesicle vaccine noted that there were 31% fewer gonococcal infections among those who received the meningococcal vaccine than in the placebo group [39].

In the mouse model, Connolly et al [38] have reported that mice receiving the meningococcal vaccine had significantly fewer gonococci recovered from the endometrium and oviduct of immunized animals compared with controls. Their study suggests that immunization accelerates clearance of gonococci from both the upper and lower genital tracts of mice. The meningococcal outer membrane vesicle has been incorporated into a commercially licensed meningococcal vaccine, Bexsero (GSK), and antibodies induced by immunization with Bexsero recognize gonococcal antigens [40]. A study has been planned to evaluate the efficacy of Bexsero to prevent gonorrhea infection in gay and bisexual men (ClinicalTrials.gov Identifier: NCT04415424). Future studies will be needed to evaluate the impact of gonococcal vaccines in women.

Chlamydial vaccines are under active development, and mouse studies have shown that chlamydial subunit vaccines combined with T-helper 1–inducing adjuvants can induce protection from genital challenge [41], and the safety and immunogenicity of a chlamydia vaccine candidate CTH522 has been evaluated in a first in human trial [42]. Having a greater understanding of host protective mechanisms for *N. gonorrhoeae* and *C. trachomatis* will be essential to develop combined vaccine approaches for prevention of PID and tubal factor infertility in women [43].

In this issue of *The Journal of Infectious Diseases,* the state of the science related to PID and female reproductive health sequelae related to STIs is described. While significant work has been accomplished to improve our understanding of populations at highest risk and agents that may be the causes of this important outcome, much work is still needed, and progress has been limited. Evidence-based prevention approaches to reducing the incidence of PID and associated morbidity are lacking, and larger well-designed studies are needed to evaluate some of the new tools for better detection of upper genital tract infection. The work highlighted in the current issue can help set the stage for the next generation of clinical, laboratory, and public health science to inform better diagnosis, clinical management, and prevention of PID.

Notes

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