Association between *Mycoplasma genitalium* and acute endometritis

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Up to 70% of cases of pelvic inflammatory disease do not have a known cause. We recruited 115 women who had presented to a clinic for sexually transmitted diseases in Nairobi, Kenya with pelvic pain that had persisted for 14 days or less, to look for an association between *Mycoplasma genitalium* and endometritis. With PCR, we detected *M genitalium* in the cervix, endometrium, or both in nine (16%) of 58 women with histologically confirmed endometritis and in one (2%) of 57 women without endometritis (p=0·02). Our results suggest that infection with *M genitalium* is strongly associated with acute endometritis in this population.

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Pelvic inflammatory disease (PID) is the most frequently diagnosed severe infectious gynaecological disorder, and can result in serious sequelae. Yet, in many cases of PID, no microbial cause can be identified. Serological investigations[1,2] of an association between PID and *Mycoplasma genitalium* have been inconclusive, and whether *M genitalium* is able to ascend into the upper genital tract and cause disease has not been established. However, results of molecular studies have shown an association between *M genitalium* and non-gonococcal urethritis[3] and cervicitis.[4]

We analysed the association of *M genitalium* infection with histologically confirmed acute endometritis. We recruited 115 women aged 18–40 years who presented with low abdominal pain that had lasted for 14 days or less at the referral clinic for sexually transmitted diseases in Nairobi, Kenya. Women were excluded if they were pregnant, breastfeeding, had taken antibiotics for 2 or more days in the preceding 2 weeks, had had surgery, had given birth, or had had an abortion in the previous 6 weeks. All women gave written informed consent, and the study was approved by the ethnical review committee at the Kenyatta National Hospital in Nairobi and the University of Washington in Seattle, WA, USA.

Using a Pipelle (Unimar, Inc, Wilton, CT, USA), we took a biopsy sample from the endometrium for histopathological testing and for PCR of *M genitalium*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* DNA. Endometrial tissue for histological assessment was stored in 10% buffered formalin and stained with haematoxylin and eosin, and methyl-green pyronin. Endometritis was defined as presence of at least one plasma cell per 120× microscopic field of endometrial stroma.[5] Cervical and endometrial samples for PCR were frozen in STM at –20°C within 3 h of collection and assayed for *N gonorrhoeae* and *C trachomatis* DNA with Cobas Amplicor (Roche Diagnostic Systems, Branchburg, NJ, USA), and for the DNA of *M genitalium* DNA by a modification of the *M genitalium* adhesion protein PCR.[3] All samples that tested positive for MgPa (*M genitalium* P1-like adhesion gene) DNA were retested with a modification of a second PCR assay[3] that targets the ribosomal DNA of *M genitalium*. Vaginal samples were cultured for *Trichomonas vaginalis* (InPouch, BioMed Diagnostics Inc, San Jose, CA, USA) and gram-stained for bacterial vaginosis. We did serological screening for HIV-1 using Detect HIV, (Biochem ImmunoSystems, Inc, Montreal, Canada), and confirmed the results with Recombigen, (Cambridge Biotech Ltd, Ireland). Peripheral blood CD4 and CD8 T-lymphocytes were counted with Facscan, (Becton Dickson Inc, Sunnyvale, CA, USA).

We analysed data using SPSS for Windows version 9.0. Univariate analysis included χ^2 and Fisher's exact tests for categorical variables, Mann-Whitney test for intervals, and Student's *t* test for continuous variables.

Of 212 women with acute pelvic pain, 163 (77%) had an endometrial biopsy sample that had an adequate amount of tissue for histological assessment. Of these, 115 (71%) had sufficient frozen endometrial tissue to test for the DNA of *M genitalium* and these women thus constituted our study population. Demographic characteristics, sexual and reproductive history, and serological and microbiological findings of women who did and did not have endometrial specimens tested for *M genitalium* did not differ

	Included (n=115)	Excluded (n=97)	p
Demographic characteristics			
Age (mean [SD], years)	26·3 (6·7)	26·3 (5·2)	0·95
Married	55 (48%)	46 (47%)	0·95
Years of education (mean, SD)	8·5 (2·8)	8·5 (2·7)	0·86
Sexual and reproductive history			
Age at first sexual intercourse (mean [SD], years)	17·6 (2·5)	17·2 (2·8)	0·32
Pregnancies (median [range])*	1 (0–8)	1 (0–9)	0·71
Current use of hormonal contraception	39 (34%)	36 (37%)	0·63
History of prostitution	5 (4%)	9 (9%)	0·15
Number of sexual partners in past 3 months (median; range)	1 (0–15)	1 (0–30)	0·30
Lifetime number of sexual partners (median; range)	3 (1 to >1000)	3 (1 to >1000)	0·06
History of similar disease	39 (34%)	27 (26%)	0·32
Clinical complaints and findings			
Complaint of abdominal pain			
Mild	82 (71%)	59 (61%)	..
Moderate	25 (22%)	33 (34%)	..
Severe	8 (7%)	5 (5%)	0·13
Time with abdominal pain (mean [SD], days)	9·8 (10·6)	8·6 (8·1)	0·37
Serological and microbiological results†			
Endometritis	58 (50%)	28/46 (58%)	0·36
HIV-1 seropositive	37 (33%)	32 (33%)	0·96
Bacterial vaginosis	38/78 (49%)	38/72 (53%)	0·62
Trichomonas vaginalis	17/103 (17%)	14/80 (18%)	0·86

Values are number (%) unless otherwise indicated. *25th and 75th percentile for those included=2 and 5, and for those excluded=3 and 6. †49 women did not have an endometrial biopsy sample taken that had sufficient tissue for histological assessment, and an additional 48 women did not have enough frozen endometrial tissue to test for *Mycoplasma genitalium* DNA.

Table 1: **Comparison of demographic, reproductive, clinical, microbiological, and serological data**

	Endometritis (n=58)	No endometritis (n=57)	p
Serological and microbiological findings			
Mycoplasma genitalium			
Cervix*	6 (12%)	1 (2%)	0·11†
Endometrium‡§	7 (12%)	0	0·01†
Either‡	9 (16%)	1 (2%)	0·02†
Neisseria gonorrhoeae			
Cervix	8 (15%)	4 (8%)	0·23
Endometrium	6 (10%)	1 (2%)	0·11†
Either	9 (16%)	4 (7%)	0·15
Chlamydia trachomatis			
Cervix§	3 (6%)	1 (2%)	0·62†
Endometrium	4 (7%)	2 (4%)	0·68†
Either	4 (7%)	2 (4%)	0·35†
HIV-seropositive	27 (48%)	10 (18%)	0·001
Bacterial vaginosis‖	17 (45%)	21 (53%)	0·49
Trichomonas vaginalis	11 (22%)	6 (11%)	0·14
Immunological results			
Peripheral blood CD4¶ T-lymphocytes/μL (mean; SD)	377 (167)	363 (211)	0·84

*No coinfections with *N gonorrhoeae* or *C trachomatis* detected· †Fisher's exact test. ‡Coinfection with *N gonorrhoeae* detected in one cervical sample, with *C trachomatis* in one patient's cervical and endometrial samples.· §n=54 for endometritis and n=53 for no endometritis. ¶Of HIV-1 positive women only, n=36. ‖n=38 for endometritis and n=40 for no endometritis.

Table 2: **Comparison of microbiological, serological, and immunological results by presence or absence of histologically diagnosed endometritis**

significantly (table 1), suggesting that restriction of the analysis to these 115 women did not bias our results.

58 (50%) of the 115 women had endometritis that was histologically confirmed (table 2). *M genitalium* was identified in ten (9%) women; four (40%) had this bacterium in both cervical and endometrial samples, and three (30%) each in cervical and in endometrial samples alone. The association between *M genitalium* and endometritis was significant whether this organism was detected in the cervix plus or minus the endometrium (p=0·02), or in the endometrium alone (p=0·01; table 2). After exclusion of samples that were coinfected with *N gonorrhoeae* and *C trachomatis*, the association between endometritis and infection with *M genitalium* in the cervix, endometrium, or both was not significant (p=0·06). However, *M genitalium* infection in the endometrium alone was associated with endometritis even after exclusion of these coinfections (p=0·03), suggesting that *M genitalium* was associated with endometritis whether or not other recognised causes were present.

Demographic, reproductive, serological, and other microbiological factors were compared with presence or absence of endometritis and *M genitalium* infection. Women with and without endometritis were close in age (26·2 [SD 5·2] *vs* 26·3 [8·0] years, p=0·94), median number of (three [IQR 1–60] *vs* three [1–30] sexual partners during their lifetime (p=0·31), and history of similar complaints (33% *vs* 37%, p=0·70). Neither *N gonorrhoeae* nor *C trachomatis* was significantly associated with endometritis (table 2), possibly because of screening and treatment programmes for these infections in this population, low statistical power due to small sample size, or both. Infection with HIV-1, but not level of immunosuppression as measured by CD4 T-lymphocyte count, was strongly associated with endometritis (odds ratio 4·3; 95% CI 1·8–10·1). Abdominal pain was reported as mild (rather than moderate or severe) by all women who were infected (either in the cervix or endometrium) with *M genitalium* by comparison with 68% of women who were not infected with this organism (p=0·06). Similarly, eight (89%) of nine women infected with *M genitalium*, compared with 59 (53%) of 102 uninfected women had easily induced cervical bleeding (p=0·08). Five (14%) of 37 women who were seropositive for HIV-1 versus five (6%) of 78 who were seronegative were infected with *M genitalium* (p=0·29). *M genitalium* infection was not associated with age, marital status, age at first sexual intercourse, or median number of sexual partners.

Although our results suggest that *M genitalium* is an important cause of endometritis, its mechanisms of action and association with disease need further investigation. The general perception of *N gonorrhoeae* and *C trachomatis* as the only important sexually transmitted causes of upper genital tract infection in women will probably need revision if further investigations lend support to our results.

Contributors

C Cohen headed the study in Nairobi, with E Bukusi as the lead clinician, and R Brunham as the main investigator. E Bukusi organised the laboratory investigations in Nairobi, which were designed by K Holmes and J Bwayo and overseen by S Sinei. S Astete and L Manhart organised the laboratory investigation in Washington, which was done with the help of K Holmes, and headed by P Totten. C Cohen, L Manhart, and R Brunham did the data analysis. C Cohen, L Manhart, S Astete, and P Totten wrote the report, with help from E Bukusi, R Brunham, K Holmes, S Sinei, and J Bwayo.

Conflict of interest statement

None declared.

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1 Moller BR, Taylor-Robinson D, Furr PM. Serological evidence implicating *Mycoplasma genitalium* in pelvic inflammatory disease. *Lancet* 1984; **1:** 1102–03.

2 Lind K, Kristensen GB. Significance of antibodies to *Mycoplasma genitalium* in salpingitis. *Eur J Clin Microbiol* 1987; **6:** 205–07.

3 Bjornelius E, Lidbrink P, Jensen JS. *Mycoplasma genitalium* in non-gonococcal urethritis—a study in Swedish male STD patients. *Int J STD AIDS* 2000; **11:** 292–96.

4 Uno M, Deguchi T, Komeda H, et al. *Mycoplasma genitalium* in the cervices of Japanese women. *Sex Transm Dis* 1997; **24:** 284–86.

5 Kiviat NB, Wolner-Hanssen P, Eschenbach DA, et al. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. *Am J Surg Pathol* 1990; **14:** 167–75.

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