GYNECOLOGY

The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology

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BACKGROUND: Chronic endometritis is a persistent inflammation of the endometrial mucosa caused by bacterial pathogens such as Enterobacteriaceae, *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Mycoplasma*, and *Ureaplasma*. Although chronic endometritis can be asymptomatic, it is found in up to 40% of infertile patients and is responsible for repeated implantation failure and recurrent miscarriage. Diagnosis of chronic endometritis is based on hysteroscopy of the uterine cavity, endometrial biopsy with plasma cells being identified histologically, while specific treatment is determined based on microbial culture. However, not all microorganisms implicated are easily or readily culturable needing a turnaround time of up to 1 week.

OBJECTIVE: We sought to develop a molecular diagnostic tool for chronic endometritis based on real-time polymerase chain reaction equivalent to using the 3 classic methods together, overcoming the bias of using any of them alone.

STUDY DESIGN: Endometrial samples from patients assessed for chronic endometritis (n = 113) using at least 1 or several conventional diagnostic methods namely histology, hysteroscopy, and/or microbial culture, were blindly evaluated by real-time polymerase chain reaction for the presence of 9 chronic endometritis pathogens: *Chlamydia trachomatis*, *Enterococcus*, *Escherichia coli*, *Gardnerella vaginalis*, *Klebsiella pneumoniae*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Staphylococcus*, and *Streptococcus*. The sensitivity and specificity of the molecular analysis vs the classic diagnostic techniques were compared in the 65 patients assessed by all 3 recognized classic methods.

RESULTS: The molecular method showed concordant results with histological diagnosis in 30 samples (14 double positive and 16 double negative) with a matching accuracy of 46.15%. Concordance of molecular and hysteroscopic diagnosis was observed in 38 samples (37 double positive and 1 double negative), with an accuracy of 58.46%. When the molecular method was compared to microbial culture, concordance was present in 37 samples (22 double positive and 15 double negative), a matching rate of 56.92%. When cases of potential contamination and/or noncultivable bacteria were considered, the accuracy increased to 66.15%. Of these 65 patients, only 27 patients had consistent histological + hysteroscopic diagnosis, revealing 58.64% of nonconcordant results. Only 13 of 65 patients (20%) had consistent histology + hysteroscopy + microbial culture results. In these cases, the molecular microbiology matched in 10 cases showing a diagnostic accuracy of 76.92%. Interestingly, the molecular microbiology confirmed over half of the isolated pathogens and provided additional detection of nonculturable microorganisms. These results were confirmed by the microbiome assessed by next-generation sequencing. In the endometrial samples with concordant histology + hysteroscopy + microbial culture results, the molecular microbiology diagnosis demonstrates 75% sensitivity, 100% specificity, 100% positive and 25% negative predictive values, and 0% false-positive and 25% false-negative rates.

CONCLUSION: The molecular microbiology method describe herein is a fast and inexpensive diagnostic tool that allows for the identification of culturable and nonculturable endometrial pathogens associated with chronic endometritis. The results obtained were similar to all 3 classic diagnostic methods together with a degree of concordance of 76.92% providing an opportunity to improve the clinical management of infertile patients with a risk of experiencing this ghost endometrial pathology.

Key words: bacterial pathogens, chronic endometritis, endometrial microbiome, histology, hysteroscopy, microbial culture, molecular microbiology diagnosis, next-generation sequencing, real-time polymerase chain reaction

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Introduction

The Human Microbiome Project has highlighted the importance of microorganisms and their genomes in human health and disease,[1] and has brought to light the value of detecting dysbiotic microbiomes to facilitate the improvement of clinical management. Chronic endometritis is a persistent inflammation of the endometrial mucosa caused by the presence of bacterial pathogens in the uterine cavity. The most common infectious agents responsible for chronic endometritis are *Enterococcus faecalis*, *Enterobacteriaceae*, *Streptococcus* species, *Staphylococcus* species, *Gardnerella vaginalis*, and *Mycoplasma* species as well as genital pathogens associated with sexually transmitted infections, such as *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*.[2,3]

AJOG at a Glance

Why was this study conducted?

Chronic endometritis diagnosis still depends on the method used. The aim of this study is to develop a new molecular method for the diagnosis of chronic endometritis, overcoming the bias of the current methods.

Key findings

Molecular microbiology can be used to improve diagnosis and management of chronic endometritis in asymptomatic infertile patients.

What does this add to what is known?

Molecular microbiology can detect bacterial pathogens causing chronic endometritis and could be useful to guide a target therapy for this ghost endometrial condition.

Chronic endometritis is often clinically silent and rarely suspected and diagnosed, although it can be accompanied by symptoms like pelvic pain, dysfunctional uterine bleeding, dyspareunia, and leukorrhea.[4] The actual prevalence in the general population is ill-defined, although it has been estimated to be between 0.8-19%.[5] Even if clinically silent, chronic endometritis has been suggested to diminish the success rates of both spontaneous and assisted reproductive technology conceptions as well as contributing to obstetric and neonatal complications.[6-12] The prevalence of chronic endometritis in infertile patients has been estimated at 2.8-39%,[9,13-19] but can be as high as 60% or 66% in women diagnosed with unexplained recurrent miscarriage or repeated implantation failure, respectively.[20,21]

The diagnosis of chronic endometritis is difficult because there are no typical clinical or ultrasound findings. Classic diagnostic techniques of chronic endometritis rely on histology, which is based on the identification of plasma cells in the endometrial stroma,[4] but this method is nonspecific and dependent on the date of the menstrual cycle when sampling occurs. Considering these limitations, hysteroscopy and microbial culture are also often used for chronic endometritis diagnosis.[3,15] Hysteroscopic diagnosis of chronic endometritis relies on subjective characteristics identified by the reproductive endoscopist such as stromal edema, focal or diffuse epithelial hyperemia, and/or the presence of micropolyps. The identification of endometrial pathogens by microbial culture is the only method that provides objective information for targeted therapy. Its use has resulted in an improvement of reproductive outcome in women with recurrent miscarriage and repeat implantation failure;[20,21] however, endometrial bacterial culture is not routinely performed because it has a long turnaround time, and not all microorganisms responsible for chronic endometritis are culturable.

To improve and personalize the state of the art for diagnosing and treating chronic endometritis, researchers must determine the identity and pathogenicity of the microbes prone to produce an endometrial infection. Molecular methods have revolutionized the detection and characterization of microorganisms in a broad range of medical fields including virology, mycology, parasitology, microbiology, and dentistry.[22] For instance, in public health, the screening of *Mycobacterium tuberculosis* by polymerase chain reaction (PCR) allows for early recognition and optimized treatment.[23] Along with conventional PCR techniques, real-time (RT)-PCR has an ever-increasing role in clinical diagnostics based on its capacity to detect difficult-to-culture bacteria and generate both qualitative and quantitative results in an accurate and rapid manner.[24] The aim of this study is to compare, in the same infertile patients, the diagnostic accuracy of the molecular microbiology tool with the traditional chronic endometritis diagnostic methods, ie, endometrial histology, hysteroscopy, and/or microbial culture, by assessing the presence of 9 specific chronic endometritis pathogens by RT-PCR and next-generation sequencing (NGS).

Materials and Methods

Study design

Endometrial samples from 113 patients subjected to chronic endometritis diagnosis using endometrial histology, hysteroscopy, and/or microbial culture were blindly evaluated for the presence of 9 chronic endometritis pathogens by RT-PCR using paired endometrial samples. Then, sensitivity and specificity of the molecular analysis and the classic diagnostic techniques were compared in 65 patients with chronic endometritis results assessed by all 3 recognized classic methods (Figure 1). In parallel, endometrial samples of negative controls based on histology and microbial culture ($n = 10$) were evaluated for the presence of chronic endometritis pathogens by molecular microbiology.

Study participants

Participants involved in this study were 21- to 53-year-old infertile patients recruited (E.C.) at the Second Unit of Obstetrics and Gynecology, Department of Biomedical and Human Oncological Science, University of Bari, Bari, Italy, undergoing in vitro fertilization treatment. Patients were diagnosed and treated for chronic endometritis using either a single or various classic diagnostic techniques. Frozen endometrial biopsy specimens were sent blindly to Igenomix SL for molecular microbiology diagnosis by RT-PCR. Also, endometrial tissue from women with negative results for chronic endometritis was analyzed using RT-PCR. This group of negative controls consisted of women undergoing surgery for benign ovarian conditions (oophorectomy for dermoid cyst with exclusion of endometriosis) or operated on due to myomas (myomectomy) ($n = 6$), and women treated with antibiotics for previous chronic endometritis with no current signs of the disease at the time of sample collection by either histology and microbial culture ($n = 4$). The ethical committee of the Second Unit of