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Gram stain as a relapse predictor of bacterial vaginosis after metronidazole treatment

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Bacterial vaginosis is the most prevalent disease of the female genital tract. In spite of various effective antibiotics in the treatment of bacterial vaginosis, its high relapse rate is a common problem. Bacterial species causing bacterial vaginosis are generally unable to be cultured by conventional methods. It is impractical and inadequate to use culture methods to guide initial treatment. Gram stain of vaginal secretion is a practical tool to establish the diagnosis of bacterial vaginosis. We enrolled 78 cases of Gram stain-proven bacterial vaginosis and tried to use Gram stain as a predictor of relapse after 1 week of treatment with metronidazole. Possible predictive factors for relapse in Gram stain were analyzed, including absence of large Gram-positive rods, presence of small Gram-negative rods, small Gram-variable rods, curved Gram-variable rods, or Gram-positive cocci. Gram stain was repeated immediately after treatment, and at 1 month and 3 months after treatment. All cases showed beneficial clinical effect after metronidazole treatment. Eighteen cases (23.1%) relapsed during the follow-up period. All 16 cases with significant Gram-positive cocci in pretreatment smears relapsed after metronidazole treatment. Presence of small Gram-negative rods, small Gram-variable rods, and curved Gram-variable rods, or absence of large Gram-positive rods did not predict relapse. Gram-positive cocci in pretreatment smear was a good predictor of relapse after metronidazole treatment.

Key words: Bacterial vaginosis, Gram's stain, metronidazole, recurrence

Bacterial vaginosis is the most prevalent vaginal disease among women of child-bearing age. The syndrome is marked by an increased milky vaginal discharge, elevated vaginal pH and the presence of an amine or fishy odor. Microbiologically, bacterial vaginosis is characterized by a shift in the vaginal flora from the dominant flora of *Lactobacillus* spp. to a mixed vaginal flora that includes *Gardnerella vaginalis*, *Bacteroides* spp., *Mobiluncus* spp., *Mycoplasma hominis,* and peptococci [1,2]. The key point of diagnosis is to demonstrate a shift of vaginal flora. In the diagnosis of bacterial vaginosis, qualitative cultures of vaginal secretions are not useful. Quantitative bacterial cultures are very difficult to perform in routine diagnosis. Clinical diagnosis using Amsel criteria may lead to underdiagnosis of bacterial vaginosis [3]. The vaginal Gram stain using Nugent or Spiegel criteria is a sensitive and practical routine diagnostic method in the outpatient clinic [3-5]. Many articles published after the Centers for Disease Control Sexually Transmitted Diseases Treatment Guidelines produced in 1989 suggest that oral metronidazole (500 mg twice daily for 7 days) is the preferred treatment for bacterial vaginosis [6-9]. However, the treatment of bacterial vaginosis can be unsatisfactory, because of the high rate of recurrence and relapse. Clinical observation demonstrated that up to 80% of women develop recurrent bacterial vaginosis within 9 months after metronidazole treatment [6]. The reasons for recurrence and relapse are not understood. It is also difficult to devise an effective strategy for prevention and prediction of recurrence and relapse. A possible cause of recurrence and relapse may be inadequate coverage of vaginal flora by metronidazole. We tried to use Gram stain as a guide to predict the likelihood of recurrence and relapse after metronidazole treatment for bacterial vaginosis.

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Materials and Methods

The study population was a subset of women, without intrauterine devices, seen at gynecologic infectious

diseases clinic at China Medical College Hospital for evaluation of vaginal discharge, during the period from August 1, 1998 to March 31, 1999. All patients enrolled received a vaginal speculum examination without lubrication. The vaginal smear for subsequent Gram staining (crystal violet solution for 1 min, Gram's iodine solution for 1 min, acetone-alcohol decolorization for 10 s or less, safranin counterstaining for 1 min) was taken from lateral vaginal fornices by rolling a swab across the vaginal wall and then onto a glass slide and air drying.

Each Gram-stained smear was evaluated for the following morphotypes under oil immersion (× 1000): large Gram-positive rods (lactobacillus morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* spp. morphotypes), curved Gram-variable rods (*Mobiluncus* spp. morphotypes), and Gram-positive cocci. Diagnosis of bacterial vaginosis was made according to the Spiegel criteria [5]. Bacterial vaginosis was present if lactobacillus morphotypes were fewer than 5 per oil immersion field and if there were 5 or more *G. vaginalis* morphotypes together with 5 or more other morphotypes (Gram-positive cocci, small Gram-negative rods, curved Gram-variable rods, or fusiforms) per oil immersion field. If 5 or more lactobacilli and/or fewer than 5 other morphotypes were present per oil immersion field, the Gram stain was considered to be normal by the Spiegel criteria.

Cases diagnosed as bacterial vaginosis based on the vaginal Gram-stain smear were enrolled into this study. Analysis of vaginal smears was recorded, including quantitation of the various morphotypes. Each morphotype was quantitated from 1+ to 4+ with regard to the number of morphotypes per oil immersion field (0, no morphotypes; 1+, less than 1 morphotype; 2+, 1 to 4 morphotypes; 3+, 5 to 30 morphotypes; 4+, 30 or more morphotypes). Enrolled patients were treated with systemic metronidazole (500 mg twice daily) for 7 days, then were advised to avoid ingestion of any antimicrobial agents in the following 3 months. Vaginal smear for Gram stain evaluation was obtained immediately after treatment, at 1 and 3 months after treatment, and from any patients returning for relapse due to abnormal vaginal discharge. Relapse was defined as bacterial vaginosis proven by a Gram-stain vaginal smear occurring anytime during the 3-month follow-up period.

Possible risk factors for relapse based on a Gram-stain smear were analyzed, including the absence of lactobacilli, presence of Gram-negative rods, Gram-variable rods, curved Gram-variable rods, or Gram-positive cocci.

Results

236 women without intrauterine devices were seen in the gynecologic infectious diseases clinic of China Medical College Hospital for evaluation of abnormal vaginal discharge during the period from August 1, 1998 to March 31, 1999. Bacterial vaginosis was diagnosed in 98 women (41.5%) based on a Gram-stain smear. Patients' enrolled were treated with systemic metronidazole (500 mg orally twice daily) for 7 days and were followed up by vaginal smears immediately after treatment, and at 1 month and 3 months post-treatment. Only 78 patients (79.6%) completed the follow-up and abstained from antimicrobial agents during the follow-up period. Their mean age was 30.7 years, with a range from 19 to 46 years.

Smear of all enrolled cases showed mixed morphotypes. The average number of morphotypes in each smear was 2.9. The morphotype distribution of the 78 enrolled cases comprised absence of large Gram-positive rods in 31 patients (39.7%); presence of small Gram-variable rods in 78 cases (100%) [= 3+]; presence of small Gram-negative rods (= 3+) in 70 cases (89.7%); presence of curved Gram-variable rods (= 3+) in 18 cases (23.1%); presence of Gram-positive cocci (= 3+) in 16 cases (20.5%) [Table 1]. At completion of metronidazole treatment, most smears showed marked reduction of bacteria flora, especially small Gram-negative rods (100% to 0%), small Gram-variable rods (87.2% to 5.1%), and curved Gram-variable rods (23.1% to 1.3%) [Table 1]. Large Gram-positive rods morphotype were unaffected by metronidazole, and no reduction was observed even when initial smears showed only 1+ to 2+. Large, Gram-positive rod morphotype, absent before treatment, was restored in 2 smears. Five smears with 1+ to 2+ large, Gram-positive rods morphotype before treatment showed significant increase to 3+ to 4+ after treatment. Gram-positive cocci morphotype in 16 pretreatment smears all persisted without reduction of bacteria number at completion of metronidazole treatment.

At 1 month after treatment, 62 cases (79.5%) showed recovery of large, Gram-positive rods to significant levels (3+ to 4+) and were defined as normal vaginal smears. Sixteen cases without significant large, Gram-positive rods morphotype (0 to 2+) were classified as

Table 1. Distribution of vaginal bacterial morphotypes in 78 cases of bacterial vaginosis before, immediately after, and 1 and 3 months after metronidazole treatment (500 mg twice daily for 7 days)

Morphotype	No. of cases (%)			
	Before treatment	After treatment (months)		
		0	1	3
No large Gram-positive rods	31 (38.7)	29 (37.2)	10 (12.8)	14 (17.9)
Large Gram-positive rods (1-2+)	47 (61.3)	44 (56.4)	6 (7.7)	4 (5.1)
Large Gram-positive rods (3-4+)	0 (0)	5 (6.4)	62 (79.5)	60 (76.9)
Small Gram-negative rods (= 3+)	78 (100)	0 (0)	1 (1.3)	2 (2.6)
Small Gram-variable rods (= 3+)	68 (87.2)	4 (5.1)	1 (1.3)	2 (2.6)
Curved Gram-variable rods (= 3+)	18 (23.1)	1 (1.3)	0 (0)	0 (0)
Gram-positive cocci (= 3+)	16 (20.5)	16 (20.5)	16 (20.5)	16 (20.5)

bacterial vaginosis, including 14 cases of predominant Gram-positive cocci morphotype and 2 cases of mixed Gram-positive cocci and other morphotypes.

At 3 months after treatment, morphotype distribution pattern was similar to that at 1 month. 60 cases (76.9%) showed significant levels (3+ to 4+) of large, Gram-positive rods and were defined as normal smears. Two cases with significant large, Gram-positive rods morphotype on the smear performed 1 month after treatment returned to bacterial vaginosis, 1 had predominantly small, Gram-negative rods morphotype, and the other small, Gram-variable rods morphotype. Sixteen cases with significant Gram-positive cocci morphotype on the vaginal smear at 1 month showed persistence without any change.

Discussion

Bacterial vaginosis is a common cause of abnormal, malodorous vaginal discharge. Metronidazole was shown to be effective in 1978 and has remained a treatment of choice for more than 2 decades. It provides a variable clinical efficacy of between 70-90%, because of the complex nature of the vaginal flora. Although metronidazole is unable to kill the full spectrum of bacterial flora presenting in bacterial vaginosis, the clinical efficacy is not absolutely dependent upon in vitro data. Bacterial interaction may contribute to the remarkable clinical efficacy of metronidazole monotherapy in the true, mixed-type infection, which includes both aerobic and anaerobic bacteria.

In this series, metronidazole effectively eliminated implicated bacteria from 73.1% (57/78) of the cases immediately after a complete treatment course. The most problematic morphotype is Gram-positive cocci. All Gram-positive cocci morphotype distributed in 16 pretreatment smears persisted and outnumbered other morphotypes (16:5) after a course of metronidazole treatment. Metronidazole is active against anaerobic organisms, both bacteria and protozoa, and a few facultative anaerobes. Organisms generally considered routinely susceptible to metronidazole are anaerobic Gram-negative bacilli, anaerobic Gram-positive bacilli, anaerobic cocci, and protozoa. It is therefore not surprising that the Gram-positive cocci morphotype in this series was not eradicated by routine metronidazole treatment, to later become a major cause of early microbiologic failure. The positive and negative predictive values of Gram-positive cocci morphotype for treatment failure are 100% (16/16) and 91.9% (57/62) at the end of treatment.

After a course of metronidazole treatment, vaginal flora are restored with large Gram-positive rods morphotype becoming a predominant species in most cases, even in cases where pretreatment smears did not show this morphotype. At the end of 1 month, vaginal smears returned to normal in 79.5% cases. Despite the gradual disappearance of Gram-negative rods and Gram-variable rods morphotypes from the vaginal smears, all 16 cases containing Gram-positive cocci morphotype in pre- and post-treatment smears persisted and became the only cause of microbiologic failure (16/16) at the end of 1 month. This means that Gram-positive cocci morphotype is the main cause and predictor of early relapse and treatment failure when patients are treated with metronidazole. Both positive and negative predictive values of Gram-positive cocci morphotype for treatment failure at the end of 1 month are 100%.

Three months after metronidazole treatment, all 16 cases containing Gram-positive cocci morphotype persisted as a cause of treatment failure. However, some cases with significant large Gram-positive morphotype at 1-month smear showed invasion by abnormal flora, such as Gram-negative and Gram-variable rods.

This may be the main cause of recurrent bacterial vaginosis after metronidazole treatment. Recurrence is not easily predicted by Gram stain of pre- and post-treatment vaginal smears. However, we believe that recurrent cases will increase with time lapse after withdrawal of metronidazole if predisposing factors of bacterial vaginosis cannot be identified and eradicated at the same time antibiotic treatment is given. The total rate of relapse and recurrence is estimated to reach 80% in literature reports if the follow-up period is long enough. The positive and negative predictive values of Gram-positive cocci morphotype for treatment failure at the end of 3 months are 88.9% (16/18) and 96.8% (60/62).

In this article, Gram-positive cocci morphotype in pretreatment smear is a good predictor of relapse from metronidazole treatment with high positive and negative predictive values. Causes of recurrence are more complicated and unpredictable. Treatment with other broad-spectrum antibiotics, such as ampicillin-sulbactam or amoxicillin-clavulanic acid for bacterial vaginosis are equally effective. Whether these antibiotics are more effective or not in the prevention of relapse is an issue deserving further study.

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