JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2000, p. 3096–3097 Vol. 38, No. 8
0095-1137/00/$04.00+0

Use of 5-Bromo-4-Chloro-3-Indolyl-α-D-*N*-Acetylneuraminic Acid in a Novel Spot Test To Identify Sialidase Activity in Vaginal Swabs from Women with Bacterial Vaginosis

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Received 28 February 2000/Returned for modification 13 April 2000/Accepted 16 May 2000

The validity of measuring vaginal sialidase activity to identify bacterial vaginosis (BV) was determined by using 5-bromo-4-chloro-3-indolyl-α-D-*N*-acetylneuraminic acid in a near-patient test. The sensitivity and specificity of the test for prediction of BV were 95.6 and 96.3%, respectively. Positive and negative predictive values were 95.6 and 96.3%, respectively. This test may be an alternative to Gram staining.

Bacterial vaginosis (BV), the vaginal condition in which commensal lactobacilli are overwhelmed by other members of the vaginal flora such as *Gardnerella vaginalis*, *Prevotella* spp., and *Mobiluncus* spp., is associated with endometritis and preterm labor (5, 8). It is postulated that the BV-related flora ascends to the uterus and causes the release of inflammatory mediators that initiate labor (3, 5, 7, 11). One potential causal mechanism is the sialidase activity produced by BV-related microorganisms. Sialidases (neuraminidases), which remove sialic acid from sialoglycoconjugates, have been significantly associated with BV (1, 2, 7, 8, 9). One group who reported a significant risk of preterm birth in women with BV found that this risk was also independently associated with sialidase activity in the vaginal secretions (8).

Given the association between sialidases, BV, and disease and the difficulty in diagnosing BV in a clinical setting, we developed a colorimetric test for sialidase using 5-bromo-4-chloro-3-indolyl-α-D-*N*-acetylneuraminic acid (BCIN) as a substrate. This communication describes the association between this test and BV.

One hundred women (median age, 28 years; age range, 16 to 52 years) who were attending a genitourinary clinic and for whom a vaginal speculum examination was necessary were studied. Women were evaluated for *Candida albicans* by microbiological culture from a high vaginal swab and trichomoniasis by wet preparation. Two high vaginal swabs were taken simultaneously after speculum insertion; one swab was used to prepare a glass slide for microscopy and the other swab was used for the sialidase spot test. This was placed immediately into a buffer composed of 2 ml of 25 mM Tris–HCl–Tween 20 (pH 7.0). The swabs in buffer were refrigerated at 4°C until the end of the clinical study.

The substrate BCIN (Rose Scientific, Edmonton, Alberta, Canada) was chosen as it gave a strong colorimetric response on filter paper to control sialidase (1 U of type X *Clostridium perfringens* sialidase; Sigma Chemical Co., St. Louis, Mo.) and BV-associated sialidase. The specificity of the substrate was validated by a conventional liquid assay with purified *C. perfringens* sialidase and BV-associated organisms and did not show any nonspecific effect of pH variation. The activity detected by BCIN correlated with that detected by using sialoglycoprotein substrates (7), and this activity could be inhibited by using the specific sialidase inhibitors 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid and cupric chloride. The substrate was prepared at 0.63 mM in 150 mM sodium acetate–25 mM CaCl–1 mM NaCl (pH 5.5) and was used to dampen a 10-cm-diameter piece of Whatman no. 1 filter paper. The swabs were removed from the buffer and were applied to the substrate-inoculated filter paper. This was placed in a covered petri dish, and the petri dish was incubated for 30 min at 37°C. A blue spot became visible in between 2 and 30 min, indicating that sialidase activity was present. In the absence of sialidase activity the paper remained colorless even after a period of 2 h, before the filter paper dried out.

Diagnosis of BV by Gram staining was assessed by use of a modification of the criteria of Spiegel et al. (10) as described by Hay et al. (4). Thus, we graded the flora as normal (grade 1; predominantly lactobacillus morphotypes), intermediate (grade 2; reduced lactobacilli mixed with other morphotypes), and BV (grade 3; few or no lactobacilli with greatly increased numbers of *G. vaginalis* and/or other morphotypes).

The intermediate category (4) and its relationship to both BV and "normal" vaginal flora needs further elucidation. This category does not fulfill the definition of BV, and it is unclear whether the intermediate grade is an indication that vaginal colonization with BV-related microorganisms undergoes a stepwise progression from normal flora through intermediate flora to BV-related flora. Hillier et al. (6) found either that the flora of 62% of women with intermediate flora "reverted" to normal flora or that the women developed BV, indicating the instability of this type of colonization. It is also possible that the flora of women with intermediate flora may revert to normal flora and that the women never develop BV or may develop BV but that the flora never reverts to either intermediate or normal flora. In this study, therefore, data for the normal and intermediate grades were pooled for the purposes of the statistical analysis. Observers were blinded to the results of the spot test, and spot test observers were blinded to the results of Gram staining.

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TABLE 1. Results of Gram staining and spot test

Vaginal flora grade	No. (%) of samples		
	Total	Sialidase positive	Sialidase negative
Normal or intermediate (grade 1 or 2)	54	2 (4)	52 (96)
BV (grade 3)	46	44 (96)	2 (4)

Groups were compared by χ^2 analysis by Fisher's exact test with Epi-Info software (version 6.01; Centers for Disease Control and Prevention, Atlanta, Ga.).

The results obtained by Gram staining and estimation of sialidase by the BCIN spot test are shown in Table 1. The floras of a total of 54 patients were of either the normal ($n = 45$) or intermediate ($n = 9$) grade. Ninety-six percent of these patients showed no positive reaction by the BCIN spot test. Two samples from the group whose flora was of the normal grade were positive by the spot test. No samples from the group whose flora was of the intermediate grade demonstrated a positive reaction by the spot test. The group whose flora was of the BV grade ($n = 46$) demonstrated a high proportion of positive spot tests (96%), with 2 (4%) of the patients whose flora was of this grade being negative for sialidase activity by this spot test. A positive spot test for sialidase activity was significantly correlated with the incidence of BV grade 3 on diagnosis by Gram staining ($P < 0.000001$). There was no correlation between the incidence of trichomoniasis or *C. albicans* and either BV or a positive spot test. The sensitivity and specificity of the spot test for the prediction of BV were high (95.6 and 96.3%, respectively). Positive and negative predictive values were 95.6 and 96.3%, respectively.

This communication describes a rapid, simple technique with a high sensitivity and specificity for the diagnosis of BV. It recognizes the enzyme sialidase, which may cause adverse obstetric and gynecological outcomes when it is produced by BV-associated flora. Sialidase, an enzyme frequently associated with pathological conditions, shows high levels of activity in pregnant and nonpregnant women with BV. Sialidase activity may act to remove sialic acids from cervical mucins and diminish the viscosity inherent in the mucus. The effects may be twofold. First, mucin and therefore mucus organization may be lost, rendering the mechanical and bacteriostatic properties of mucin less effective as barrier mechanisms. Second, exposure of cryptic structures in oligosaccharides can promote bacterial adhesion to the secreted mucus and the underlying epithelial glycocalyx. This may create conditions for bacterial invasion of the upper reproductive tract. Identification of sialidase activity by the spot test in patients with BV may therefore be beneficial for two reasons. First, it can be undertaken in the same room as the patient, as it does not require the use of light microscopy for Gram staining. Second, as sialidase activity is not always present in patients with BV, its presence may be an indicator that the condition is more detrimental to the host in these cases. This possibility requires further investigation.

We thank the staff of the Milne Clinic, Bristol Royal Infirmary, for assistance in carrying out this study.

We also thank Tommy's Campaign, London, United Kingdom, for funding the project (grants 30 and 49).

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