

# Direct or Referral Microscopy of Vaginal Wet Smear for Bacterial Vaginosis: Experience from an STD Clinic

CARSTEN SAND PETERSEN<sup>1</sup>, ANNE GRETHE DANIELSEN<sup>1</sup> and JAN RENNEBERG<sup>2</sup>

Departments of <sup>1</sup>Dermato-venereology and <sup>2</sup>Microbiology, Bispebjerg Hospital, University of Copenhagen, Denmark

**A new wet smear diagnostic criterion for bacterial vaginosis was applied to 124 consecutive female patients attending an STD clinic located in the centre of Copenhagen. Bacterial vaginosis was detected in 54 (44%) women, making bacterial vaginosis the most prevalent pathological condition encountered. A total of 47 (87%) of the women were symptomatic. Concomitant genital infections were found in 13 (24%) of these women, most often as vulvo-vaginal candidiasis. A correct microscopic diagnosis could also be obtained by sending the vaginal smear to the local microbiologist for rehydration and phase contrast microscopy. It is suggested that the previously described vaginal wet smear criteria are used in place of Amsel's criteria for routine diagnosis of bacterial vaginosis. Key words: bacterial vaginosis; diagnosis; vaginal wet smear.**

(Accepted May 19, 1999.)

Acta Derm Venereol 1999; 79: 473–474.

Carsten Sand Petersen, MD, Department of Dermato-venereology, Bispebjerg Hospital, DK-2400 NV Copenhagen, Denmark.

Bacterial vaginosis (BV) is the main cause of vulvo-vaginal complaints among women attending general practitioners (1, 2) and out-patients gynaecological clinics (3). During the last decade significant changes have occurred in the prevalence of sexually transmitted diseases in a large STD clinic in Copenhagen, making BV the most common cause of vaginal discharge, itching and vulval irritation (4).

The composite clinical criteria of Amsel et al. (5, 6) have been recommended as the diagnostic reference standard, with the presence of at least 3 or 4 characteristics: a homogeneous grey-white discharge; pH value >4.5; release of fishy amine odour on addition of potassium hydroxide; and the presence of clue cells on microscopy (5). It has been shown that the specificity of a homogeneous vaginal discharge is low (7). The amine test ("sniff test") is also a subjective component, making the classical diagnostic criteria of BV less suitable. Recently it has been demonstrated that the 4 components Amsel et al. showed considerable variation of association, whereas a simple wet smear criterion, evaluating the presence of lactobacilli morphotypes, small bacteria morphotypes and clue cells was more consistent and accurate in diagnosing BV (8).

We have applied this new, rapid and easily performed vaginal wet smear criterion for diagnostics of BV in both asymptomatic women and women presenting with various genital complaints. Phase contrast microscopy was conducted in the STD clinic, but in addition a vaginal smear was sent to the local microbiological section for direct microscopy after rehydration, enabling us to compare the results of the microscopic findings in the clinic with those obtained in the reference microbiological department.

## PATIENTS AND METHODS

In 1997 a total of 124 consecutive women referred to our STD clinic were examined for the presence of BV using a simple vaginal wet smear criterion, as described previously (8). Vaginal secretion from the fornix posterior was visualized by phase contrast microscopy at 400× magnification immediately and a vaginal smear was dried and sent to the local microbiological centre for phase contrast microscopy after rehydration with isotonic saline, as described previously (9). A microscopic appearance consistent with BV was based on identifica-

Table I. Clinical manifestations in 124 consecutive female patients attending an STD clinic in Copenhagen

Clinical manifestation	n
Asymptomatic	47
Symptomatic	77
Vaginal discharge	52
Itching/irritation	16
Abdominal pain	3
Various complaints <sup>a</sup>	6

<sup>a</sup>Includes unpleasant smell (n=1), irregular bleeding (n=1), coital pain (n=1), cystitis-like symptoms (n=1) and painful labial swelling (n=1).

Table II. Final diagnosis in 124 consecutive female patients attending an STD clinic in Copenhagen in 1997

Final diagnosis	n
Bacterial vaginosis <sup>a</sup>	54
Vulvo-vaginal candidiasis <sup>a</sup>	16
Chlamydia	12
Genital warts	6
Trichomoniasis <sup>a</sup>	3
Molluscum contagiosum	1
Genital herpes	1
Salpingitis	1
No abnormal findings	38
Total	132

<sup>a</sup>Diagnosis based on direct microscopy of vaginal wet smear.

Table III. Number of female patients with symptomatic and asymptomatic genital infection according to final diagnosis

Final diagnosis	Symptomatic	Asymptomatic
Bacterial vaginosis (n=54)	47	7
Vulvo-vaginal candidiasis (n=16)	12	4
Chlamydia (n=12)	5	7
Trichomoniasis (n=3)	2	1
Gonorrhoea (n=2)	1	1
Total	67	20

Table IV. Results of phase contrast microscopy of vaginal secretion from 124 females for bacterial vaginosis (BV) performed in the STD clinic and by a local microbiologist

		Microscopy by venereologist		
		Positive BV criteria	Negative BV criteria	Total
Microscopy by microbiologist	Positive BV criteria	42	7	49
	Negative BV criteria	5	70	75
	Total	47	77	124

tion of clue cells, lack of lactobacilli and predominance of coccobacilli including *Gardnerella vaginalis* and motile rods (8). Consistency was calculated using kappa statistics (10). At the same time, all women were tested for gonorrhoea, chlamydia, trichomoniasis, *Gardnerella vaginalis* and vulvo-vaginal candidiasis, based on standard routine procedures (4). The clinical manifestations are shown in Table I.

## RESULTS

The results are shown in Tables II–III. BV was the most prevalent pathological condition encountered in the study group (Table II). As shown, it was associated with genital complaints in the majority of the women (Table III).

A comparison of the rate of positive BV diagnoses in the STD clinic and the referral microbiological department is shown in Table IV. Consistency in microscopic BV diagnoses was obtained in 112 (93%) of 124 patients. Nine of 12 patients with inconsistent microscopic findings had malodorous discharge, significant growth of *Gardnerella vaginalis* and a clinical response to oral treatment with metronidazole. None of the 70 women with negative microscopy for BV in the STD clinic and microbiological department had symptoms or signs suggesting BV. The concordance between the 2 observers were almost perfect, with a kappa value of 85 (95% CI 0.68–0.91)

Concomitant infections were diagnosed in 13 (24%) of the 54 women with BV. These were: vulvo-vaginal candidiasis ( $n=7$ ); chlamydia ( $n=4$ ); trichomoniasis ( $n=4$ ); and a combination of gonorrhoea, chlamydia and trichomoniasis ( $n=1$ ). A total of 29 (70%) of the 41 women with isolated BV were symptomatic.

## DISCUSSION

The diagnoses of BV in this study were based on previously described vaginal wet smear microscopic criteria, with a sensitivity and specificity comparable to the criteria of Amsel et al. (5) and Gram stain criteria (11). The results in this study demonstrate that the diagnoses of BV in women attending an STD clinic could be based on the characteristics in a vaginal wet mount. Clue cells are easily identified as vaginal epithelial cells so massively coated with coccobacilli that the cell borders are obliterated, *Lactobacillus* morphotypes are seen as large non-motile, rods and small bacteria morphotypes as small, non-motile, pleomorphic coccobacillary forms (8). Lactobacilli were assessed as lacking when there were fewer than 5 per field.

It is concluded that Amsel's composite criteria may be

replaced in the STD clinic by these more simple and rapid vaginal wet smear criteria. If a microscopic service is not at hand in the clinic it seems adequate to obtain a BV microscopic diagnosis the following day by sending a dry vaginal smear to the local microbiological department for examination. Dermato-venereologists or general practitioners who see too few patients with vaginal discharge to obtain sufficient training in phase contrast microscopy may therefore choose to send samples of vaginal secretions to the local microbiologist for direct microscopy of BV. The presence of candida spores and hyphae can be evaluated at the same time.

## REFERENCES

- McCue JD. Evaluation and management of vaginitis. An update for primary care practitioners. *Arch Intern Med* 1989; 149: 565–568.
- Wathne B, Holst E, Hovelius B, Mardh PA. Vaginal discharge – comparison of clinical, laboratory and microbiological findings. *Acta Obstet Gynecol Scand* 1994; 73: 802–808.
- Fleury FJ. Adult vaginitis. *Clin Obstet Gynecol* 1981; 24: 407–438.
- Petersen CS, Danielsen AG, Renneberg J. Bacterial vaginosis – the leading cause of vaginal discharge in women attending an STD-clinic in Copenhagen. *Acta Derm Venereol* 1999; 79: 414.
- Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; 74: 14–22.
- Westrom L, Evaldson G, Holmes KK, van der Meijden W, Ryander E, Frederiksson B. Taxonomy of Vaginosis. Bacterial vaginosis – a definition. Reports from working groups at the symposium on bacterial vaginosis. Stockholm, 1984; 259–266. Almquist and Wiksell International, Stockholm, Sweden.
- Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* 1988; 158: 819–828.
- Schmidt H, Hansen JG. A wet smear criterion for bacterial vaginosis. *Scand J Prim Health Care* 1994; 12: 233–238.
- Larsson PG, Platz-Christensen JJ. Enumeration of clue cells in rehydrated air-dried vaginal wet smears for the diagnosis of bacterial vaginosis. *Obstet Gynecol* 1990; 76: 727–730.
- Fleiss JL. In: Statistical methods for rates and proportions. 2nd edn. New York: John Wiley and Sons Inc., 1981; 121–236.
- Schmidt H, Hansen JG. Four vaginal flora patterns assessed by wet mount microscopy. Second International Meeting on Bacterial vaginosis. The Gant Aspen, Colorado. September 17–19, 1998.