Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis)
A practical guide

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SUMMARY Anaerobic vaginosis (non-specific vaginitis) can be readily diagnosed at the time of first attendance without recourse to expensive and time-consuming laboratory investigations. Diagnosis is based on careful history-taking, clinical examination, and simple investigations in the clinic. Although the presence of a malodorous vaginal discharge and the finding of clue cells on microscopy (in the absence of Trichomonas vaginalis) are sufficient for the diagnosis, a positive amine test and a vaginal pH of more than 5·0 are useful confirmatory tests.

Introduction

In spite of a wealth of recent reports on non-specific vaginitis there is a lack of practical advice to those working in genitourinary medicine and related fields as to how this diagnosis may be made at the time of the first examination. The guidelines suggested in this report resulted from a study started at St Thomas's Hospital, London, in 1979 into the clinical and microbiological aspects of non-specific vaginitis. Details of the bacteriological and other findings are published elsewhere.1-4 The population in this study (which continues) includes patients attending the department of genitourinary medicine and a local family planning clinic. The number of patients investigated has been necessarily limited by the constraints imposed by full anaerobic culture and identification of organisms in each case and to date includes 130 women.

Non-specific vaginitis is ill-named for three reasons. Firstly, it may be confused with non-specific genital infection; secondly it is associated with the presence of defined (or definable) bacteria; and, thirdly, it is not characterised by inflammation of the vagina. There have been waves of enthusiasm for the role of Gardnerella (née Haemophilus) vaginalis as the causative organism, and some agile arguments have been put forward to explain away its poor in-vitro sensitivity to metronidazole in the face of this antibiotic's manifest clinical effect.

In 1980 Spiegel et al.5 carried out a detailed analysis of the microbial flora associated with non-specific vaginitis. They considered that anaerobes might well act synergistically with G vaginalis to give rise to the condition, and many are now suspicious that the anaerobic component is responsible for some, if not most, of the clinical symptoms and signs. For the above reasons we prefer the term "anaerobic vaginosis" as a more apt and descriptive name for the disease.

Our practical guidelines beg the question of the relative roles of G vaginalis and anaerobic bacteria in the pathogenesis of this condition and are independent of the need for expensive and time-consuming laboratory investigations. The equipment needed is already available in most departments of genitourinary medicine and comprises a bright-field microscope with ×40 and ×100 objectives, facilities for Gram-staining, pH paper (Whatman narrow range pH 4-6), 5-10% potassium hydroxide, and wooden orange sticks for mixing.

Anaerobic vaginosis

The symptoms and findings of anaerobic vaginosis include:

1. Genital malodour, often described as 'fishy'.
2. Vaginal discharge, which may vary from scanty to profuse, is often grey or grey-green in colour, and tends to be homogeneous, watery, and often frothy. There is not usually any vulval soreness or irritation.
3. The pH is 5 or greater and the amine test is positive.
(4) Clue cells are usually present in both wet mount preparation and Gram-stained slides.
(5) Lactobacilli are scanty or absent.
(6) Polymorphonuclear leucocytes are rare on both wet mount and stained preparations.
(7) Anaerobic bacteria (usually in association with G vaginalis) can be found with appropriate culture techniques.

**STEPS IN DIAGNOSIS**

**History-taking**

Although patients commonly complain of a smelly discharge anaerobic vaginosis can be a condition of minimal symptoms and signs. The patient may notice that symptoms vary with the stage of the menstrual cycle and complain that the menstrual blood has an abnormal odour. The character of the discharge may also vary at different times in the cycle, the progesterone-dominated latter part, for instance, giving rise to thicker, less frothy vaginal secretions. This sequence of events can give the patient and the examining doctor a false impression of spontaneous cure. It is therefore important to note the date of the last menstrual period, contraceptive method used (if any), and to ask whether symptoms alter in relation to the cycle.

The odour associated with this syndrome is often worse after sexual intercourse. Chen et al 6 suggested that the relatively alkaline semen reacting with the woman's infected vaginal secretions to release volatile amines. The physician should ask if the odour is worse after intercourse as this is a helpful sign.

**Clinical examination**

The discharge associated with anaerobic vaginosis is watery and non-irritant. The presence of a greyish discharge at the introitus is suggestive of the diagnosis, particularly if there is no vulval erythema. Some women with this condition have a vaginal discharge which is less than normal. If this is not a result of vaginal douching, it is a relevant clinical sign. More commonly, the patient will have a grey discharge which is homogeneous, often frothy, and tends to stick to the vaginal wall in an evenly spread coat. The vaginal mucosa has a normal appearance after the discharge has been swabbed away.

The presence or absence of vaginal odour should be noted. This can be identified either by sniffing the speculum after withdrawal (as suggested by Gardner and Dukes5) or by carrying out the sensitive amine test.

**CLINICAL INVESTIGATIONS**

**pH measurement**

The pH of the vagina in anaerobic vaginosis is >5.8 that is, less acid than normal vaginal secretions. We find narrow range (4-6) pH paper suitable. Since menstrual fluid and cervical mucus tend to raise the pH both these factors should be borne in mind when the findings are assessed. A short piece of pH paper can be clipped on to forceps and dipped into the vaginal discharge, or the paper may be placed in contact with secretions on the speculum after it has been withdrawn.

**Amine test**

This test, first described by Pheifer et al 8 involves mixing the suspect vaginal discharge with an alkali, potassium hydroxide. It is both sensitive and specific if carried out as shown in fig 1. The test result is positive if an ammoniacal odour is released when potassium hydroxide is mixed with the discharge. It works equally well with secretion which has been left to dry on a microscope slide and, once appreciated, is subsequently easily recognised. The release of the odour is transient—hence the need to mix directly under the nose. Positive amine test results are also found in most patients harbouring Trichomonas vaginalis owing to the concomitant anaerobic component of the discharge. In agreement with K K Holmes (personal communication, 1982) we occasionally find a weakly positive amine test result when spermatozoa are present in the healthy vagina.

**Microscopy**

The wet mount preparation, which is examined initially with the x 40 objective, consists of a drop of vaginal discharge mixed with a drop of normal saline and topped with a coverslip. Although not common practice it is possible to use the x 100 oil immersion objective with a drop of oil on top of the coverslip. This is of particular help in identifying vibrios and will occasionally show unexpected trichomonads.
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which, if in small numbers, may be missed by low-power examination. The Gram-stained slide, which is routinely examined using the high-power objective for the presence of *Candida albicans*, will also show vaginal epithelial cells and any associated bacteria. The wet mount and stained slide together give information on (a) clue cells; (b) leucocytes; (c) lactobacilli; (d) vibrios; and (e) spermatozoa.

Clue cells—These vaginal epithelial cells with bacteria adhering to their surface are the hallmark of anaerobic vaginosis. They have a characteristic appearance on the wet mount (figs 2 and 3). The cell has a granular appearance with bacteria blurring the epithelial cell margins. The Gram-stained slide (fig 4) shows that these bacteria are chiefly Gram-negative or Gram-variable coco-bacilli. Scattered Gram-positive organisms may also be seen but Döderlein's Gram-positive lactobacilli are conspicuous by their absence. In a recent series of 40 patients with anaerobic vaginosis initially diagnosed on history and clinical examination clue cells and a positive amine test result were found in all cases and each diagnosis was confirmed by subsequent culture of *G vaginalis* and anaerobes (Blackwell et al, unpublished data).

False-positive clue cells—In some cases lactobacilli stick to desquamated vaginal epithelial cells. If the wet mount alone is examined the doctor may be misled into a diagnosis of anaerobic vaginosis. The pH is low, however, the amine test result negative, and the stained slide shows the organisms to be Gram-positive rods (fig 5).

False-negative clue cells—Occasionally a patient with a florid, malodorous vaginal discharge of high pH will have no clue cells on the wet mount. Levinson et al9 suggested that some women, perhaps those chronically infected, produced local IgA which blocked attachment of bacteria to the cell wall. In such cases the stained slide will show masses of Gram-negative to Gram-variable coco-bacilli, most of which do not adhere to the cell wall. The pH and amine test are as for anaerobic vaginosis.

**FIG 2** Clue cell as seen using darkfield condenser. Note the presence of a relatively normal cell (b) immediately adjacent to the clue cell (a). Rafts of bacteria (c) are present in the surrounding medium together with numerous curved vibrios.
FIG 3  Clue cell as seen using × 10 eye-piece and × 100 oil immersion objective under direct light microscopy. Note the blurred cell outline (a) and circular artifacts (b) produced by corkscrew movement of bacteria during exposure.

FIG 4  Gram-stained preparation of a clue cell (a) showing adherent bacteria which are chiefly Gram-negative coco-bacilli. Rafts of bacteria (b) are seen around the clue cell and Gram-negative curved bacilli (c) can be distinguished (anaerobic vibrios).
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Polymorphonuclear leucocytes—Large numbers of leucocytes are rarely found in anaerobic vaginosis. If present the possibility of a coincidental chlamydial, gonococcal, or trichomonal infection should be suspected (see below).

Vibrios—There have been reports recently of an association of vibrios with non-specific vaginitis.3,10,11 Examination of the wet preparation with the ×100 objective shows that these organisms are of two types. The larger, some 3μ in length, has a distinctive motion (not unlike that of a spermatozoon) and shows as a Gram-negative crescent on the Gram-stained slide. The smaller vibrio is one-third the size and is Gram-variable (figs 3 and 4). It is important to distinguish between vibrios and the effect of Brownian motion on other bacteria.

Spermatozoa—The presence of spermatozoa may explain the presence of a watery, grey, slightly malodorous discharge in an asymptomatic patient without clue cells. They may also help to distinguish between treatment failures and reinfections.

TREATMENT
In 1978 Pheifer et al8 published results of an unblinded randomised trial which suggested that metronidazole was the most effective treatment for non-specific vaginitis. Since 1980, when our preliminary results concerning the bacteriology of the condition confirmed the importance of anaerobes, we have used metronidazole (400 mg twice daily for five days) to treat the condition. Early results of a single dose of 2 g metronidazole4 indicate that this regimen, which is satisfactory in the treatment of T vaginalis, is inadequate for anaerobic vaginosis.

ASSESSMENT OF CURE
The disappearance of clue cells from the wet mount preparation after treatment should be enough to confirm therapeutic success; there is also a dramatic change in the appearance of the Gram-stained slide (figs 4 and 6). The absence of Gram-negative bacteria and their replacement by lactobacilli is evidence of bacteriological cure and correlates well with symptomatic relief.

MIXED INFECTIONS
Anaerobic vaginosis is often found in conjunction with other sexually transmitted diseases. In such cases there may be an alteration in clinical symptoms, signs, and microscopical findings.
Of other vaginal infections, C. albicans is not often found with anaerobic vaginosis. When the two infections do coincide, however, there may be soreness and irritation and examination shows candidal plaques floating in the foul-smelling anaerobic discharge. The amine test result will be positive but the pH may give atypical results. Microscopy should show classical clue cells together with candidal spores or mycelia or both.

T. vaginalis has an almost invariable anaerobic component to the discharge and G. vaginalis is also found in most cases. Since the treatment for both conditions can be identical, the other findings become academic once trichomonads have been found.

Clue cells and an anaerobic discharge are frequently found in the presence of gonorrhoea. Of 200 consecutive cases of gonorrhoea in women attending St Thomas’s Hospital early in 1982, 36% were found to have anaerobic vaginosis as against 22% with trichomoniasis. Likewise, 35% of women attending as sexual contacts of men with non-specific urethritis had clue cells. The discharge in these cases may not be typical but the pH is high, the amine test result is positive, and microscopy usually shows increased leucocytes in addition to clue cells.

**Discussion**

The spate of recent reports and review articles on non-specific vaginitis, G. vaginalis, and anaerobic infection is evidence enough of the renewed interest in these conditions over the last few years. In spite of excitement about the sexually transmitted component in such bizarre conditions as Kaposi’s sarcoma and Pneumocystis carinii pneumonia, it is likely that for purely numerical reasons anaerobic vaginosis will be the sexually transmitted disease of the ’80s.

Even having adopted the pragmatic approach advocated here in terms of diagnosis, there remain considerable problems in terms of management. Does one accept that anaerobic infection is always sexually transmitted? Should the sexual partners of affected women be treated? If so, should this be on the basis of a history of contact alone or should attempts be made to establish evidence of infection in the male partner? There is a pressing need for research designed to answer these questions.

Our early experience of examining male contacts of women with anaerobic vaginosis gives some pointers. In the absence of attempts to culture anaerobes we have found G. vaginalis in the urethra...
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of some 30% of male contacts and would expect this figure to increase as sampling techniques improve. Perhaps more interestingly, almost 40% of such contacts, although asymptomatic, satisfy our microscopical criteria for the diagnosis of non-gonococcal urethritis (Kohiyar, unpublished observations). These patients are chlamydia-negative. If anaerobic culture techniques were applied with as much vigour to the male urethra as they have been in the past to the vagina some light might possibly be thrown on cases of chlamydia-negative urethritis.

We would like to express our thanks to Miss E Taylor, Alan Fox and Professor I Phillips, Department of Microbiology, and T Brandon, Department of Medical Photography, St Thomas's Hospital, and to May and Baker for financial support.

References


