BACTERIAL VAGINOSIS

(clue cell-positive discharge)
Diagnostic, ultra-structural and therapeutic aspects

Bacteriële Vaginose (clue cell-positive discharge)
Diagnostische, ultra-structurele en therapeutische aspecten

Proefschrift

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.Dr. A.H.G. Rinnooy Kan
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
vrijdag 4 september 1987 om 15.45 uur

door

Willem Idaniël van der Meijden

geboren te Brielle

1987
Van Gorcum, Assen/Maastricht

Promotiecommissie

Promotor: Prof.Dr. A.C. Drogendijk
Promotor: Prof.Dr. E. Stolz
Overige leden: Prof.Dr. J. Huisman
Prof.Dr. P. Piot

De handelseditie van dit proefschrift verschijnt bij uitgeverij *Van Gorcum* te Assen/Wolfbero, New Hampshire, U.S.A., onder ISBN 90 232 2309 8.

To Helen, Ido and Walter
To my parents and parents in law

'Vaginitis is the most common disease of the female genital tract and therefore is of great social, economic, and medical importance'

Herman L. Gardner (1912-1982)

Contents

I INTRODUCTION

Vaginal discharge may be defined as 'loss of fluid other than blood or urine from the introitus'. It is a common reason for women to seek medical attention.

The word 'leucorrhoea' is often misused. Since it indicates the flow of a white substance, it should only be used to describe an excessive amount of normal secretions. Vaginal secretions consist of exfoliated vaginal and cervical epithelial cells, cervical mucus, transudate (including leucocytes) and bacteria. Normal discharge usually makes the introitus feel moist, but will only occasionally stain the underclothing. It will, however, be increased under certain circumstances, some of which are hormone-dependant (e.g. periovulatory period, pregnancy). Abnormal vaginal discharge is mostly of infectious origin and can be associated with pruritus vulvae, vulvar soreness and dyspareunia.

Adequate examination of a woman complaining of vaginal discharge requires the taking of the history, an appropriate physical examination and relevant laboratory techniques.

There is substantial evidence that the great majority of vaginal infections are caused by the presence of *Trichomonas vaginalis*, *Candida* species and *Gardnerella vaginalis* plus anaerobes. *T. vaginalis* was described as early as 1836 by Donné[1], but for a long time was regarded as a harmless commensal. Hoehne[2] was the first to link the presence of the protozoan to purulent vaginitis. Vaginal fungi were linked to vaginitis as early as 1849[3].

It was not until 1955 that a third type of infection, commonly referred to as 'non-specific' bacterial vaginitis, received the recognition which its clinical importance appeared to warrant. Gardner and Dukes[4] presented evidence that the vast majority of these vaginitides constitute a specific infectious entity, the presence of which is suggested by 'a grey, homogeneous, odorous discharge having a pH of 5.0 tot 5.5'. These investigators learned to place great reliance on the appearance of wet mounts. They introduced the term 'clue cell', referring to epithelial cells with a characteristic granular appear-

ance and indefinite outlines and stated that 'a correct diagnosis could be made in practically every instance on the basis of the wet mount findings alone'.

In the past thirty years the condition, originally known as *Haemophilus vaginalis* vaginitis[4], has been the subject of many studies. They often yielded contradictory results as to its cause and clinical manifestations. The original view of a single aetiological agent, namely *H.vaginalis*, later renamed *G.vaginalis*[5], had to be abandoned. Pheifer et al.[6] were the first to hypothesize that anaerobes play a role in the pathogenesis of the condition. Its polymicrobial origin has since been repeatedly confirmed, both by anaerobic cultures and gas-liquid chromatography. Since *G.vaginalis* can also be isolated from normal secretions[7,8], most authors agree that the diagnosis should not be based on the presence of this micro-organism. There has recently been a renewed interest in the anaerobic curved rods, first described by Curtis[9]. They only seem present together with other anaerobes and *G.vaginalis* and can be isolated from 50% of women with 'non-specific' vaginitis[10]. While these bacteria may merely constitute a part of the anaerobic mixture, Larsson et al.[11] proposed a causal connection between the rods and bleeding complications.

The presence of clue cells can be considered pathognomonic of 'non-specific' vaginitis[12]. Spiegel et al.[13] preferred to base the diagnosis on the presence of at least two of the following criteria: homogeneous consistency, vaginal pH>4.5, positive amine test ('fishy' odour upon addition of potassium hydroxide) and clue cells in the wet mount preparation. The fact that only a minority of epithelial cells seem covered with bacteria is commented on by some authors. Fleury[14] originally thought that *G.vaginalis* preferred cells with higher glycogen content but later found that this was not the case.

Until recently many terms were used to designate the clinical entity. Since the condition is a specific vaginal disease not accompanied (or only very seldom) by vaginitis, the term 'non-specific vaginitis' had to be discouraged. The term 'bacterial vaginosis' (BV), which was introduced a few years ago[15], seems more appropiate. Van der Meijden[16] proposed the term 'clue cell-positive discharge' (CCPD), which is based on the clue cell as characteristic feature of the condition. He also stated that 'use of the term CCPD reduces the risk that authors might discuss different conditions and thus facilitates future analyses'. BV is said to be the most common vaginal infection[17,18]. In a study among unselected female university students BV was diagnosed in 25%[7]. Studies among women visiting sexually transmitted diseases (STD) clinics report even higher percentages, up to 64%[19].

Evidence of sexual transmission of *G.vaginalis* is circumstantial. The majority of male sex partners of women with BV harbour the micro-organism in the urethra[4,6]. Since it is not a cause of urethritis, its presence is probably transient. It can, however, be associated with balanoposthitis[20].
The question whether or not BV should be considered a STD is still unanswered. On the one hand, BV seems limited to women who are sexually active[7] and sexual abuse in children can be followed by BV[21]; on the other hand, treatment of the male partners does not seem to prevent recurrences[22,23]. Several investigators proposed an association between BV and the use of an IUD[7,24]. It was postulated that the IUD determines whether the woman carrying *Gardnerella* is going to develop the syndrome[25]. The role of other factors associated with sexual intercourse, e.g. mechanical irritation of the vaginal wall and disturbance of the vaginal pH by semen, is as yet unclarified.
BV is generally a clinically mild disease without complications. There have been some reports on *G.vaginalis* bacteremia in obstetrical and gynaecological patients[26,27]. However, most of the patients recovered regardless whether antimicrobial therapy directed against *G.vaginalis* was or was not given. Recently an association was proposed between BV and prematurity[28].
The treatment of BV has long been controversial and has recently been reviewed by Piot[29]. Topically applied sulphonamide cream was widely used following the initial description of BV[4]. Its efficacy, however, seems low. The use of ampicillin and tetracycline is only moderately effective and also predisposes to vaginal candidosis. The introduction of metronidazole for this condition can be considered a break-through. Pheifer et al.[6] reported a cure rate of 98% with a regimen of 2x500 mg for 7 days. There are contradictory reports on the efficacy of a single dose of 2 g metronidazole[30,31]. Høvik[32] reported good efficacy of 2 g metronidazole on day 1 and day 3. It was recently shown that the clinical efficacy and activity against *G.vaginalis* of Augmentin® is comparable with that of metronidazole[33]. Preliminary data indicate that BV can also be effectively treated with quinolones[34]. Povidone-iodine is reported to have a low efficacy. However, it causes symptomatic relief[35,36]. Insertion of lactate-gel into the vagina, 5 ml daily for 7 days, is reported to be as effective as metronidazole[37]. At present, routine male treatment cannot be advocated. This should be reserved for recurrent disease[22].
Data on the natural course of BV are scarce and contradicting. While Gardner and Dukes[4] allowed some patients to go untreated for as long as four months and none showed spontaneous cure or alteration in the clinical picture, other authors report spontaneous cures in more than half the

cases[36,38].

The abundance of medical data is in sharp contrast with the virtual absence of data on women's attitudes towards vaginal discharge, their need for information, the use of alternative treatment and the major reasons of patient delay. The impression exists that women who consult their doctor only represent the tip of the iceberg.

References

1. Donné MA. Animalcules observés dans les matières purulentes et le produit des sécrétions des organes genitaux de l'homme et de la femme. Comptes Rendus Hebdomadaire des Séances de l'Académie des Sciences 1836; 3: 385-386.
2. Hoehne O. *Trichomonas vaginalis* als haüfiger Erreger einer typischen Colpitis purulenta. Zbl Gynäkol 1916; 40: 4-15.
3. Wilkinson JS. Some remarks upon the development of epiphytes. Lancet 1849; 2: 448-451.
4. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified 'nonspecific' vaginitis. Am J Obstet Gynecol 1955; 69: 962-976.
5. Greenwood JR, Pickett MJ. Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus, *Gardnerella: G.vaginalis* (Gardner and Dukes) comb.nov. Int J Syst Bacteriol 1980; 30: 170-178.
6. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. New Engl J Med 1978; 298: 1429-1434.
7. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983; 74: 14-22.
8. Masfari AN, Duerden BI, Kinghorn GR. Quantitative studies of vaginal bacteria. Genitourin Med 1986; 62: 256-263.
9. Curtis AH. A motile curved anaerobic bacillus in uterine discharges. J Infect Dis 1913; 12: 165-169.
10. Blackwell AL, Phillips I, Fox AR, Barlow D. Anaerobic vaginosis (non-specific vaginitis): clinical, microbiological and therapeutic findings. Lancet 1983; *ii*: 1379-1382.
11. Larsson P-GB, Bergmann BB. Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? Am J Obstet Gynecol 1986; 154: 107-108.
12. Piot P, Van Dijck E, Godts P, Vanderheyden J. A placebo-controlled, double-blind comparison of tinidazole and triple sulfonamide cream for the treatment of nonspecific vaginitis. Am J Obstet Gynecol 1983; 147: 85-89.
13. Spiegel CA, Amsel RA, Eschenbach D, Schoenknecht F, Holmes KK. Anaerobic bacteria in nonspecific vaginitis. New Engl J Med 1980; 303: 601-607.
14. Fleury FJ. The clinical signs and symptoms of *Gardnerella*-associated vaginosis. Scand J Infect Dis 1983; suppl 40: 71-72.
15. Spiegel CA, Davick P, Totten PA, Chen KCS, Eschenbach DA, Amsel R, Holmes KK. *Gardnerella vaginalis* and anaerobic bacteria in the etiology of bacterial (nonspecific) vaginosis. Scand J Infect Dis 1983; suppl 40: 41-46.

16. Van der Meijden WI. Clinical aspects of *Gardnerella vaginalis*-associated vaginitis. A review of the literature. Scand J Urol Nephrol 1985; suppl 86: 135-141.
17. Fleury FJ. Adult vaginitis. Clin Obstet Gynecol 1981; 24: 407-438.
18. Vontver LA, Eschenbach DA. The role of *Gardnerella vaginalis* in nonspecific vaginitis. Clin Obstet Gynecol 1981; 24: 439-460.
19. Embree J, Caliando JJ, McCormack WM. Nonspecific vaginitis among women attending a sexually transmitted diseases clinic. Sex Transm Dis 1984; 11: 81-84.
20. Kinghorn GR, Jones BM, Chowdhury FH, Geary I. Balanoposthitis associated with *Gardnerella vaginalis* infection in men. Brit J Vener Dis 1982; 58: 127-129.
21. Hammerschlag MR, Cummings M, Doraiswamy B, Cox P, McCormack WM. Nonspecific vaginitis following sexual abuse in children. Pediatrics 1985; 75: 1028-1031.
22. Eschenbach DA. Vaginal infection. Clin Obstet Gynecol 1983; 26: 186-202.
23. Swedberg J, Steiner JF, Deiss F, Steiner S, Driggers DA. Comparison of single-dose vs one-week course of metronidazole for symptomatic bacterial vaginosis. JAMA 1985; 254: 1046-1049.
24. Kivijärvi A, Järvinen H, Grönroos M. Microbiology of vaginitis associated with the intrauterine contraceptive device. Brit J Obstet Gynaecol 1984; 91: 917-923.
25. Proceedings of the First International Conference on Vaginosis (Ed.Csángó PA), page 124. Almquist & Wiksell Periodical Company, Stockholm, 1983.
26. Venkataramani TK, Rathbun HK. *Corynebacterium vaginale* (*Haemophilus vaginalis*) bacteremia: clinical study of 29 cases. John Hopkins Med J 1976; 139: 93-97.
27. Reimer LG, Reller LB. *Gardnerella vaginalis* bacteremia: a review of thirty cases. Obstet Gynecol 1984; 64: 170-172.
28. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986; 67: 229-237.
29. Piot P. Bacterial vaginosis. An evaluation of treatment. Scand J Urol Nephrol 1985; suppl 86: 229-235.
30. Balsdon MJ. Treatment of the *Gardnerella vaginalis* syndrome with a single 2 gram oral dosage of metronidazole. Scand J Infect Dis 1983; suppl 40: 101-102.
31. Blackwell A, Fox A, Phillips I, Barlow D. Metronidazole in treatment of non-specific vaginitis. Scand J Infect Dis 1983; suppl 40: 103-106.
32. Høvik P. Nonspecific vaginitis in an outpatient clinic. Comparison of three dosage regimens of metronidazole. Scand J Infect Dis 1983; suppl 40: 107-110.
33. Symonds J, Biswas AK. Amoxycillin, augmentin, and metronidazole in bacterial vaginosis associated with *Gardnerella vaginalis*. Genitourin Med 1986; 62: 136.
34. Blenk H. Treatment of bacterial vaginosis with ciprofloxacin. Book of Abstracts, p.401. Vth Mediterranean Congress of Chemotherapy, Cairo, 26 October - 1 November 1986.
35. Fleury FJ. *Gardnerella*. Changing patterns of management. Female Patient 1981; 6: 44-47.
36. Dattani IM, Gerken A, Evans BA. Aetiology and management of non-specific vaginitis. Brit J Vener Dis 1982; 58: 32-35.
37. Andersch B, Forssman L, Lincoln K, Torstensson P. Treatment of bacterial vaginosis with an acid cream: a comparison between the effect of lactate-gel and metronidazole. Gynecol Obstet Invest 1986; 21: 19-25.
39. Bump RC, Zuspan FP, Buesching WJ, Ayers LW, Stephens TJ. The prevalence, six-month persistence, and predictive values of laboratory indicators of bacterial vaginosis (nonspecific vaginitis) in asymptomatic women. Am J Obstet Gynecol 1984; 150: 917-924.

II STUDY OBJECTIVES

This thesis deals with several aspects of (abnormal) vaginal discharge, focusing especially on clue cell-positive discharge (bacterial vaginosis, non-specific vaginitis). It reports data on epidemiology and clinical features, pathogenesis, and treatment of this vaginal disease entity, as well as on women's experience of troublesome vaginal discharge.

Chapter III discusses the question whether or not symptoms and signs have a discriminatory ability in the diagnosis of specific vaginal infections. If so, this could help the physician to reach a diagnosis with minimum effort. However, data from the literature indicate that this ability is limited[1,2]. While bacterial vaginosis and trichomoniasis undoubtedly have much in common, e.g. the overgrowth of anaerobic bacteria[3,4], the question is whether the difference between the two entities ends with the pathognomonic presence of clue cells and trichomonads, or whether other discriminating features can be detected. Chapter IV reports on an extensive clinical and laboratory study of women with these vaginal infections. The available information on the extent of the problem of vaginal discharge is incomplete. Hard figures, which probably represent only the tip of the iceberg, are being collected by several sentinel stations[5]. Data on views of women on vaginal discharge and their behaviour in this context are virtually lacking. In order to find answers to some questions, a survey was held among 5,900 women. It is reported in chapter V.

Wet mount microscopy of vaginal discharge from women with clue cell-positive discharge (CCPD) invariably shows that a minority of vaginal epithelial cells (VECs) are covered with bacteria, a finding also reported by other authors[6]. Chapter VI studies this 'selective' bacterial adherence and focuses on vitality and glycogen content of VECs, as well as on the morphology of bacterium-VEC interaction.

Chapter VII evaluates the efficacy of single-dose tinidazole in the treatment of non-specific vaginitis. Metronidazole, another nitroimidazole combination, has been shown to be very effective in a regimen of 2x500 mg for one week[7]. From the point of view of patient compliance and cost effectiveness single-dose treatment seems preferable. Although there is no convincing evidence that metronidazole has teratogenic properties in human beings[8], the drug should be used only reluctantly during pregnancy. Since CCPD is perhaps the most prevalent vaginal infection, the development of an effective treatment which can be safely used during pregnancy seems important. In chapter VIII the efficacy of amoxycillin and amoxycillin plus clavulanic acid in the treatment of CCPD is compared with that of metronidazole. Povidone-iodine has been used for local treatment of vaginitis since the early Sixties. Its vaginal use nowadays mainly concerns the treatment of chronic, non-specific vaginal infections. Most studies report moderate efficacy (up to 70%) of povidone-iodine, administered either as pessary, solution or gel. Only one study seriously questions the efficacy of povidone-iodine as a vaginal therapeutic[9]. Chapter IX concerns a double-blind, placebo-controlled study of the efficacy of povidone-iodine in the treatment of CCPD.

References

1. Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980; 141: 137-143.
2. Bergman JJ, Berg AD, Schneeweiss R, Heidrich FE. Clinical comparison of microscopic and culture techniques in the diagnosis of *Candida* vaginitis. J Fam Pract 1984; 18: 549-552.
3. Piot P, Van Dijck E, Godts P, Vanderheyden J. The vaginal microbial flora in non-specific vaginitis. Eur J Clin Microbiol 1982; 1: 301-306.
4. Brockmann J, Höhne C. Bakteriologische Aspekte der Trichomonaden Kolpitis. Zbl Gynäkol 1979; 101: 722-726.
5. Lamberts H. Morbidity in general practice. Diagnosis related information from the Monitoring Project. Utrecht: Uitgeversmaatschappij Huisartsenpers B.V., 1984.
6. Vontver LA, Eschenbach DA. The role of *Gardnerella vaginalis* in nonspecific vaginitis. Clin Obstet Gynecol 1981; 24: 439-460.
7. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. New Engl J Med 1978; 298: 1429-1434.
8. Roe FJC. Toxicologic evaluation of metronidazole with particular reference to carcinogenic, mutagenic, and teratogenic potential. Surgery 1983; 93: 158-164.
9. Dattani IM, Gerken A, Evans BA. Aetiology and management of non-specific vaginitis. Br J Vener Dis 1982; 58: 32-35.

III SOME ASPECTS OF THE DIAGNOSIS OF SPECIFIC VAGINAL INFECTIONS IN THE ROTTERDAM STD CLINIC POPULATION*

Willem I.van der Meijden[1], Paul I.M.Schmitz[2], Aat C.Drogendijk[3], and Ernst Stolz[1]

Summary

We studied specific vaginal infections in 351 consecutive women who visited the Rotterdam STD clinic. Prostitutes less often had vaginal or vulvar complaints than non-prostitutes (Fisher test, $p>0.01$), but more often had abnormal discharge (Fisher test, $p>0.001$). Normal secretions were found in 52% of the women. Clue cell-positive discharge (CCPD) was by far the most prevalent vaginal disease entity (32%). The wet mount showed pseudohyphae in 14 women (4%) and *Trichomonas vaginalis* was detected in 20(6%).

Microscopy (normal saline, no KOH 10%) had an overall sensitivity of 18% in the diagnosis of vaginal yeast infection. The sensitivity substantially rose with the 'degree' of infection. In the case of trichomoniasis the sensitivity was 55%.

The rates of positive cultures of *Candida* species, *T.vaginalis*, *N.gonorrhoeae* and *C.trachomatis* were 21%, 13%, 9% and 10% respectively.

Symptoms and signs were not of much help in the correct classification of the different diagnostic categories due to considerable overlap. However, curdy secretions are indicative of candidal infection. Tests for anaerobic overgrowth showed a 99% correct classification of normal secretions, which

* Submitted for publication (Scand J Infect Dis)

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands

[2] Institute of Biostatistics, Erasmus University, Rotterdam, The Netherlands

[3] Department of Obstetrics and Gynaecology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands

implies that smelling the vaginal secretions on the withdrawn speculum is a major diagnostic office procedure.

Introduction

Genitourinary symptoms such as vaginal discharge, malodour, dysuria and vulvar pruritus, are among the most common reasons for women to seek medical attention. These symptoms present the physician with a significant diagnostic challenge. If she or he has had adequate training, the management of vaginal infections especially can be a gratifying part of the office gynaecology. With improper methods, however, repeated treatment failures are a continuing source of frustration to both patient and doctor.
The immediate question for the physician is whether any combination of symptoms predicts diagnosis accurately enough so that one or more diagnoses can be eliminated from consideration at the outset[1]. Evidence is mixed. While some investigators consider the symptoms of the three most common vaginal infections, i.e. bacterial vaginosis (recently indicated as clue cell-positive discharge[2]), vaginal candidosis and vaginal trichomoniasis, 'rather specific'[3], most authors believe that there is considerable overlap between the symptoms of the different clinical syndromes. This indicates that symptomatology is of only minor importance in establishing the diagnosis. There is also often a notorious discrepancy between the complaint of discharge and its objective assessment by trained observers[4].
Since only a minority of women with vaginal candidosis and trichomoniasis show the classic signs of these disease entities, i.e. white, curdy secretions and yellow-green, frothy discharge[5,6], it would seem apparent that a definite diagnosis cannot be made on the basis of the gross appearance of the vaginal secretions.
In the diagnosis of candidal and trichomonal infections, microscopy of the wet mount preparation is far less sensitive than culture[7,8], especially in the case of an asymptomatic carrier. However, direct microscopy is still looked upon as a major diagnostic tool in the office situation.
The Rotterdam sexually transmitted diseases (STD) clinic provides facilities for STD control free of charge. Promiscuous persons, especially prostitutes, visit the clinic at regular intervals (1-4 times monthly). The finding of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* invariably leads to treatment, while in the case of positive candidal cultures treatment is only given in the presence of specific clinical findings and/or complaints. The impression is that clue cell-positive discharge (CCPD) is a

prevalent and frequently recurring condition among prostitutes. In these women treatment is instituted only in the case of troublesome discharge. Our main objective was to investigate the discriminatory ability of defined symptoms and signs in determining the presence or absence of specific vaginal infections as diagnosed by microscopy of the wet mount preparation.

Patients and methods

Study population. We enrolled all consecutive women who visited the outpatient department of venereology during the study period. There were no exclusion criteria. For each woman the data of only one visit were included.
Biographic data. Apart from the age of the woman and whether or not she was employed as a prostitute, we recorded data concerning use of contraceptives, use of condoms (prostitutes), practice of vaginal irrigation as well as date of last sexual intercourse, presence of complaints related to vaginal infection and the specific reason for encounter.
Gynaecological examination. This was always done by the same observer (vdM), with the woman in the lithotomy position. The external genitalia were examined, with special emphasis on signs of vulvitis (redness and/or oedema), presence of ano-genital warts and presence of discharge in the vaginal vestibule. After insertion of a lubricated bi-valve speculum attention was paid to the main characteristics of vaginal discharge (amount, consistency, colour, frothiness) and the gross aspects of cervix and vagina. The odour of the vaginal secretions was determined by smelling the discharge present on the withdrawn speculum. Malodour was assumed to indicate anaerobic overgrowth[9]. We further measured the pH of the discharge by means of indicator paper, range 4.0-7.0 pH(Merck, Darmstadt, FRG). The amine test was done by dripping some KOH 10% on the lower valve of the speculum and mixing it with the discharge present. When a rotten fish odour arose it was recorded as positive. Uterus and adnexae were palpated in the case of lower-abdominal complaints.
Specimen collection. During first visits of both prostitutes and non-prostitutes an extensive microbiological study was performed. We took swabs for cultures of *N. gonorrhoeae* (cervix, urethra), *C. trachomatis* (cervix), *T. vaginalis* (posterior fornix) and *Candida* species (lateral vaginal wall). The routine culturing in prostitutes comprises cultures for *N.gonorrhoeae* and *T.vaginalis*, while *C.trachomatis* is generally cultured once a month. During subsequent visits of non-prostitutes, cultures were performed inconsistently. We did not take cultures for *Gardnerella vaginalis*

and anaerobes. A wet mount preparation was made in all women by mixing a loopful of discharge (posterior fornix) with a loopful of normal saline.

Direct microscopy. This was performed in the office by examining the wet mount at a magnification of 400x (≤10 microscopic fields). We paid attention to the presence of leucocytes, Döderlein's bacilli, clue cells, trichomonads and pseudo-hyphae. We did not make use of KOH 10% in the diagnosis of vaginal yeast infection. The microscopical data were the basis of one of six possible diagnoses: 1.normal secretions, 2.vaginal candidosis, 3.clue cell-positive discharge (CCPD), 4.vaginal trichomoniasis, 5.trichomoniasis + clue cells and 6.'indefinite', the criteria for which will be discussed later.

Bacteriological techniques. N.gonorrhoeae strains were isolated and identified on a selective gonococcal agar medium according to standard procedures. *C.trachomatis* was cultured on a Hela 229 monolayer which was grown in a 96-well microtitre tissue culture plate (Falcon no.3070). After inoculation the plates were incubated for 48 hours at 37°C in a CO_2 incubator. Detection of chlamydial inclusions was carried out with the aid of fluorescein monoclonal antibodies (SYVA inc.). *T.vaginalis* was cultured in Ringer-Cysteine-Liver-Glucose medium for three days under aerobic conditions at 37°C. *Candida* species were cultured on Sabouraud's agar for seven days under aerobic conditions at 25°C.

Tentative and definite diagnoses. After each of several data clusters (i.e. symptoms, signs and tests for anaerobic overgrowth) a tentative diagnosis was made, largely on the basis of empirical grounds. Examination of the wet mount led to the definite diagnosis (Table I). The exclusive presence of Döderlein's bacilli was assumed to indicate normal secretions, while CCPD or trichomoniasis were diagnosed in the case of the presence of clue cells or *T.vaginalis*. The presence of both these indicators at the same time was also recorded. When pseudo-hyphae were seen, the diagnosis was vaginal candidosis. In the case of absence of the abovementioned microscopical parameters, we recorded the diagnosis as 'indefinite'.

Statistical methods. Comparison of percentages was performed using Fisher's exact test (for two percentages) or a X^2 test (for three or more percentages). For determination of the discriminatory ability of symptoms and signs, Fisher's linear discriminant analysis was used[10,11]. From the classification matrix the percentages of correct classification (accuracy) were calculated, for each diagnostic category as well as overall.

Table I. Criteria for tentative and definite diagnoses in the clinical and microscopical study of vaginal discharge

	tentative diagnosis	definite diagnosis
Symptoms		
none	normal	-
profuse discharge	'indefinite'	-
malodorous discharge	CCPD	-
profuse and malodorous discharge	trichomoniasis	-
pruritus and/or dyspareunia and/or dysuria	candidosis	-
Signs		
floccular discharge	normal	-
curdy discharge	candidosis	-
pasty discharge	CCPD	-
watery and white discharge	normal	-
watery and other than white discharge (no blood)	trichomoniasis	-
Tests for anaerobic overgrowth		
non-odorous discharge	normal	-
malodorous discharge		
- 4.4 <pH≤5.5	CCPD	-
- pH >5.5	trichomoniasis	-
Direct microscopy (wet mount)		
Döderlein's bacilli	-	normal
clue cells	-	CCPD
trichomonads	-	trichomoniasis
trichomonads and clue cells	-	trichomoniasis + clue cells
pseudo-hyphae	-	candidosis
none of above	-	'indefinite'

Results

A total of 351 women were examined; 124(35%) of them were registered as prostitutes. The mean age of the study group was 29.4 yr(± 8.3).
Prostitutes used contraception significantly more often than non-prostitutes, especially oral contraceptives (Table II); 93 prostitutes (75%) occasionally worked with a condom, while 26 women (21%) stated that they never worked without. Five women(4%) never used condoms at all. Vaginal irrigation was practised by 75% of the prostitutes, a diluted Betadine® iodine solution being most often used.
As shown in Table III, the majority of the women(61%) did not have vaginal

Table II. Demographic data on study population

	prostitutes (n=124)	non-prostitutes (n=227)	total (n=351)
Mean age ± SD	30.6 ± 6.9	28.8 ± 9.0	29.4 ± 8.3
Contraception			
pill	78(63)	94(41)***	172(49)**
IUD	5(4)	16(7)	21(6)
condom	2(2)	12(5)	14(4)
pessary	0(0)	4(2)	4(1)
female sterilization	28(22)	25(11)**	53(15)
male sterilization	0(0)	2(1)	2(1)
none	11(9)	74(33)***	85(24)

( ) number in parentheses = percentage
** $0.001<p<0.01$, Fisher test
*** $p<0.001$, Fisher test

or vulvar complaints, while prostitutes were more often without complaints than non-prostitutes ($p<0.01$). Profuse discharge appeared to be the major complaint (29%), followed by pruritus/irritation (16%) and malodorous discharge (14%). The prevalence of the different vaginal disease entities is shown in Table IV. CCPD was significantly more common among prostitutes ($p<0.05$). There were no significant differences between the prevalences of candidosis and trichomoniasis. Normal secretions were more often found in non-prostitutes ($p<0.001$).

Table III. Presence of complaints related to vaginal infection

	prostitutes (n=124)	non-prostitutes (n=227)	total (n=351)
Complaints			
profuse discharge	29(23)	73(32)	102(29)
malodorous discharge	16(13)	35(15)	51(14)
pruritus/irritation	11(9)	45(20)**	56(16)
dysuria	8(7)	30(13)	38(11)
dyspareunia	3(2)	14(6)	17(5)
No complaints	89(72)	125(55)**	214(61)

( ) number in parentheses = percentage
** $0.001<p<0.01$, Fisher test

Table IV. Results of wet mount microscopy and bacterial cultures in study population

	prostitutes (n=124)	non-prostitutes (n=227)	total (n=351)
Microscopy			
CCPD only	51(41)	61(27)*	112(32)
trichomoniasis only	3(2)	8(4)	11(3)
candidosis only	2(2)	11(5)	13(4)
trichomoniasis + clue cells	7(6)	2(1)*	9(3)
candidosis + clue cells	1(1)	0(0)	1(0.3)
normal secretions	45(36)	137(60)***	182(52)
no diagnosis ('indefinite')	15(12)	8(4)	23(7)
Cultures#			
Candida species	10/ 49(20)	24/114(21)	34/163(21)
T.vaginalis	20/117(17)	11/126(9)	31/243(13)
N.gonorrhoeae	12/117(10)	10/126(8)	22/243(9)
C.trachomatis	4/ 44(9)	9/ 92(10)	13/136(10)

( ) number in parentheses = percentage

#data represent ratio: $\frac{\text{number of positive cultures}}{\text{number of cultures taken}}$

* $0.01<p<0.05$, Fisher test

** $0.001<p<0.01$, Fisher test

*** $p<0.001$, Fisher test

Bacterial cultures did not show significant differences between prostitutes and non-prostitutes. In total, *Candida* species, *T.vaginalis*, *N.gonorrhoeae* and *C.trachomatis* were isolated in 21%, 13%, 9% and 10% respectively. The 'overall' sensitivity of wet mount microscopy in the detection of vaginal yeast was 18% (of 34 positive cultures pseudo-hyphae were seen in six cases). When comparing microscopy with the semi-quantitative results of culture, sensitivity was 0%(0/8), 0%(0/11), 20%(1/5) and 50% (5/10) in the case of sporadic growth and growth grade 1, grade 2 and grade 3 respectively. In case of trichomoniasis, the wet mount had a sensitivity of 55% (17/31).

As shown in Table V, a considerable percentage of the women with a candidal or trichomonal infection did not have complaints. Women with only a positive culture (i.e. no pathogens detectable at microscopy) were more often without complaints than those who had a positive wet mount. However, the differences were not statistically significant.

An overview of symptoms, signs and laboratory findings in women allocated to the different diagnostic categories is presented in Table VI. We only highlight some of the data. Vaginal trichomoniasis most often led to complaints, especially profuse and malodorous discharge. The combined presence of trichomonads and clue cells, however, was less frequently associated with complaints, the rate being comparable with that in women with CCPD 'sec'. Pruritus and dysuria were the major complaints of women with candidosis. Vulvitis was seen in about one-third of women with candidosis or trichomoniasis, while it was virtually absent in the other groups. Vaginitis was a prominent finding in women with trichomoniasis (55%), but was absent in all women who had both trichomonads and clue cells. Moderate to profuse discharge was most often encountered in women with trichomoniasis or candidosis. Scanty discharge was noted in the majority of women with normal secretions and in those with CCPD, those with both trichomonads and clue cells, and those diagnosed as 'indefinite'. The great majority of women had white or whitish-yellow discharge. Yellowish-white or yellowish-green secretions were almost exclusively found in women with trichomonads present. The vaginal discharge was indicated as 'watery' in 15 of 20 women(75%) with trichomonads, in 41% of women with CCPD and in 50% of those 'indefinite'. Pasty secretions were most frequently seen in women with CCPD. A frothy discharge was present in half the women with trichomonads and in 24% of cases of CCPD. Vaginal malodour was significantly associated with the presence of clue cells or trichomonads. It was absent in women with normal secretions and in those with candidosis.

Table V. Symptomatology in women with candidal or trichomonal infection, diagnosed by microscopy of wet mount or by culture

	candidal infection		trichomonal infection	
	wet mount+ve (n=13)	culture+ve (n=28)	wet mount+ve (n=20)	culture+ve (n=14)
profuse discharge	6(46)	7(25)	12(60)	5(36)
malodorous discharge	1(8)	4(14)	7(35)	4(29)
pruritus/irritation	9(69)	13(46)	6(30)	2(14)
dysuria	7(54)	7(25)	6(30)	1(7)
dyspareunia	2(15)	3(11)	2(10)	0(0)
no complaints	5(38)	18(64)	7(35)	7(50)

( ) number in parentheses = percentage

Table VI. Symptoms, signs and laboratory findings in women with normal secretions or a specific vaginal infection*, as diagnosed by microscopy of the wet mount preparation

	normal secretions (n=182)	candidosis (n=13)	CCPD (n=112)	trichomoniasis (n=11)	trichomoniasis + clue cells (n=9)	'indefinite' (n=23)
Symptoms						
none	129(71)	5(39)	71(63)	2(18)	6(67)	14(61)
profuse discharge	43(24)	5(39)	35(31)	9(82)	3(33)	6(26)
malodorous discharge	15(8)	0(0)	24(21)	5(46)	2(22)	4(17)
pruritus/irritation	27(15)	9(69)	9(8)	4(36)	2(22)	5(22)
dysuria	17(9)	7(54)	6(5)	3(27)	3(33)	2(9)
dyspareunia	10(6)	2(15)	3(3)	2(18)	0(0)	0(0)
Signs						
Vulvitis	5(3)	4(31)	2(2)	3(27)	0(0)	1(4)
Discharge in vaginal vestibule	26(14)	5(39)	38(34)	8(73)	3(33)	5(22)
Venereal warts	14(8)	2(15)	12(11)	2(18)	0(0)	1(4)
Vaginitis	2(1)	4(31)	4(4)	6(55)	0(0)	1(4)
Amount of discharge						
none	4(2)	0(0)	2(2)	0(0)	0(0)	2(9)
scant	136(76)	6(46)	70(64)	2(18)	7(78)	17(74)
moderate	36(20)	4(31)	34(31)	8(73)	2(22)	2(9)
profuse	4(2)	3(23)	3(3)	1(9)	0(0)	2(9)
Colour of discharge						
white	116(64)	6(46)	46(41)	2(18)	1(11)	9(43)
whitish-yellow	47(26)	6(46)	42(38)	5(46)	7(78)	7(33)
yellowish-white	1(0.5)	0(0)	7(6)	3(27)	1(11)	1(5)
yellowish-green	0(0)	0(0)	2(2)	1(9)	0(0)	0(0)
blood-stained**	18(10)	1(8)	15(13)	0(0)	0(0)	4(19)
Consistency of discharge						
floccular	125(69)	6(46)	4(4)	1(9)	0(0)	5(23)
curdy	4(2)	7(54)	1(1)	0(0)	0(0)	1(5)
pasty	18(10)	0(0)	61(55)	2(18)	2(22)	5(23)
watery	33(18)	0(0)	45(41)	8(73)	7(78)	11(50)
Frothiness	5(3)	0(0)	26(24)	5(46)	5(56)	1(4)
Tests for anaerobic overgrowth						
Malodorous discharge (assessed by observer)	1(0.5)	0(0)	93(83)	8(73)	8(89)	5(22)
Vaginal pH						
≤4.4	157(88)	10(77)	8(7)	2(18)	0(0)	4(18)
>4.4	22(12)	3(23)	100(93)	9(82)	9(100)	18(82)
Amine test						
negative	180(99)	13(100)	14(13)	3(27)	0(0)	16(70)
positive	2(1)	0(0)	90(81)	3(27)	6(67)	4(17)
doubtful	0(0)	0(0)	7(6)	5(46)	3(33)	3(13)
Leucocytes (Wet mount)						
–	18(10)	0(0)	9(8)	0(0)	0(0)	1(4)
+	103(57)	3(23)	58(52)	3(27)	4(44)	10(46)
++	61(34)	10(77)	45(40)	8(73)	5(56)	11(50)

Table VI continued
Bacterial cultures#

	normal secretions (n=182)	candidosis (n=13)	CCPD (n=112)	trichomoniasis (n=11)	trichomoniasis + clue cells (n=9)	'indefinite' (n=23)
Candida species						
sporadic	6/75(8)	0/6(0)	2/61(3)	0/7(0)	0/6(0)	0/8(0)
grade 1	10/75(13)	0/6(0)	1/61(2)	0/7(0)	0/6(0)	0/8(0)
grade 2	0/75(0)	1/6(17)	3/61(5)	0/7(0)	1/6(17)	0/8(0)
grade 3	3/75(4)	5/6(83)	2/61(3)	0/7(0)	0/6(0)	0/8(0)
T.vaginalis	4/99(4)	1/7(14)	5/99(5)	9/10(90)	9/9(100)	3/18(17)
N.gonorrhoeae						
cervix	3/99(3)	0/7(0)	12/99(12)	1/10(10)	1/9(11)	3/18(17)
urethra	3/99(3)	0/7(0)	8/99(8)	1/10(10)	0/9(0)	2/18(11)
C.trachomatis	4/57(7)	0/5(0)	7/59(12)	2/4(50)	0/3(0)	0/8(0)

( ) number in parentheses = percentage

#data represent ratio: $\frac{\text{number of positive cultures}}{\text{number of cultures taken}}$

* in only one woman were both clue cells and pseudo-hyphae detected (not presented)
* * in all cases but one it concerned women with menstrual blood loss

Table VII. Percentages of correct classification (accuracy)

	Diagnostic categories				
set of classification variables*	normal secretions (n=182)	candidosis (n=13)	CCPD (n=112)	trichomoniasis ±clue cells (n=20)	overall (n=327)
symptoms (a)	84(66)	62(69)	20(5)	20(35)	57(48)
signs (b)	81(78)	54(54)	80(55)	35(70)	77(69)
tests for anaerobic overgrowth (c)	99(99)	n.a.	81(54)	40(45)	89(79)
(a)+(b)+(c)	97	62	80	55	87

( ) number in parentheses =observer's accuracy
* see Table I (observer) and Table VI (linear discriminant analysis)
n.a. = not applicable

Abnormal discharge, not including candidosis, was associated with an elevated vaginal pH(>4.4) in 80-100% of the cases. While the amine test was positive in 81% of women with CCPD, it was recorded 'doubtful' in a considerable percentage of women with trichomonads.
The results of discriminant analysis are summarized in Table VII. The analysis was performed using three separate sets of classification variables, namely: only symptoms, only signs, only tests for anaerobic overgrowth. When tested'overall', correct classification (accuracy) was highest with the use of tests for anaerobic overgrowth (vaginal odour and pH, amine test). Addition of symptoms and signs did not improve overall accuracy (89% versus 87%). Determination of the absence or presence of signs of anaerobic overgrowth was especially useful in the classification of normal secretions (99% accuracy) and somewhat less in that of CCPD (81%). Symptoms have the lowest overall accuracy (57%). They only seem of help in the correct classification of normal secretions (84%). Symptoms have a low accuracy in cases of CCPD and trichomoniasis (20%). The overall accuracy of signs is substantially higher than that of symptoms but does not reach the level of tests for anaerobic overgrowth. Its accuracy is highest in cases of normal secretions (81%) and CCPD (80%).

Discussion

In our study population we distinguished between prostitutes (known or strongly suspected to be) and non-prostitutes. Since we did not ask for the number of sexual partners however, one could argue that this distinction is rather arbitrary. Given the fact that only 21% of the prostitutes always used condoms and considering the growing concern about the spread of the human immuno- deficiency virus, it seems extremely important to stress the promotion of 'safe sex' in prostitution.
The finding that complaints were much less often present among prostitutes is perhaps explained by the frequent practice of irrigation. Psychological factors, which will not be discussed further, could also be important.
It is interesting to see that there were only minor differences in the microscopical and bacteriological data of prostitutes and non-prostitutes, Bell et al., studying STD in females in a juvenile detention centre, found that the prevalence of genital infections among prostitutes and non-prostitutes was similar[12]. This could mean that the degree of promiscuity or indirect promiscuity (having sexual intercourse with a promiscuous partner) does not influence the rate of STD to a great extent. It could also implicate, however,

that while prostitutes are at a higher risk of acquiring STD (numerous sexual contacts in general), their preventive measures (e.g. irrigation with microbicidal agents, use of condom) are more or less successful. In our study CCPD was more prevalent among prostitutes. Since the question whether or not CCPD should be considered a sexually transmitted disease entity is still unanswered, it could well be that mechanical factors (e.g. irritation of the vaginal wall by frequent intercourse) play an important role. The more or less continuous disturbance of the vaginal pH by semen should also be taken into consideration.The fact that CCPD was more often not treated in prostitutes could also be of influence. The overall prevalence rate we found (32%) is in agreement with that reported by Hill et al[13]. The prevalence rates of vaginal infection with *Candida* species and *T.vaginalis*, 21% and 13% respectively, are comparable with those found in other studies[13,14].
The rates of positive cultures of *N.gonorrhoeae* and *C.trachomatis*, 9% and 10% respectively, are considerably lower than the figures of about 20% from other STD clinics[14,15]. There are several possible reasons for this difference, the main reason probably being patient selection. Since we did not restrict selection to women presenting for the first time, it could be that prevalence rates have been influenced by recent treatment. Recently an association was proposed between the use of oral contraceptives and the isolation of *C.trachomatis*[14]. Our results seem to support this. While 'only' half the women from whom cultures were taken used the pill, 12 of 13 positive cultures (92%) were found among users. The 'overall' sensitivity of the wet mount in the detection of vaginal yeast was only 18%. Other studies reporting on an unselected population, i.e. women with and without urogenital complaints, also comment on the relatively low sensitivity of direct microscopy[7,16]. We found evidence that sensitivity rises with the 'degree' of candidal infection and that symptomatology is more pronounced in women with a positive wet mount than in those who only have a positive culture. In the case of trichomoniasis the wet mount proved to be more sensitive (55%). This is in good agreement with the results of other studies[6,7]. As in the case of *Candida*, women with a positive wet mount more often had symptoms than women with only a positive culture. This probably has to do with a larger number of trichomonads in women with a positive wet mount. It is undoubtedly true that, in the diagnosis of both candidal and trichomonal infections, results of microscopy vary with the time spent in slide examination. Since probably no physician will have enough time to spend 2 or 3 minutes on microscopy, we have arbitrarily chosen for examination of a maximum of 10 high power fields.
Clue cells were present in nine of 20 women (45%) with trichomonads in the

wet mount. Gardner and Dukes possibly were the first to comment on the simultaneous presence of *T. vaginalis* and clue cells. They found clue cells in 14% of the women with trichomoniasis but also stated that in the case of active *T. vaginalis* infection, clue cells are easily overlooked[17]. Symptoms and signs were more often mild in the case of a combined presence of *T. vaginalis* and clue cells than in the case of trichomoniasis 'sec', an observation which was not reported earlier. The numbers in both groups are small, however and further comparative studies are needed.

While some studies suggest a (strong) correlation between vaginal trichomoniasis and infection with *N. gonorrhoeae*, reporting positive cultures in 35-50% of the cases[6,8,18], we found a positive gonococcal culture in only three of 20 women (14%) with trichomoniasis. The relatively low prevalence rate of gonorrhoea in our series could be responsible.

Our study has shown that vaginal symptoms are generally poor predictors of vaginal infections. Bergman and Berg studied the usefulness of symptoms in the diagnosis of *Candida* vaginitis and concluded that all genitourinary symptoms, individually and in combinations, do not predict the diagnosis and that 'the telephone diagnosis and treatment of *Candida* vaginitis by symptoms would be inaccurate'[19]. So far as signs are concerned, curd-like secretions provide evidence of yeast vaginitis, while the possibility of trichomoniasis or CCPD is low. This has also been reported by others [20]. It is obvious that one achieves greatest accuracy by performing tests for anaerobic overgrowth, especially so far as normal secretions are concerned. Both linear discriminant analysis and the observer reached an accuracy of 99%. Smelling the secretions on the withdrawn speculum is extremely helpful in distinguishing between normal and abnormal discharge. However, one should bear in mind that the secretions associated with a candidal infection are mostly non-odorous. In clinical practice microscopy should be performed in all cases of malodorous discharge in order to establish whether it is CCPD, or trichomoniasis, or both. In the case of non-odorous secretions microscopy to search for pseudo-hyphae is indicated only in the presence of specific complaints such as vulvar pruritus and dysuria, or a peculiar curdy aspect of the secretions.

References

1. Berg AO, Soman MP. Lower genitourinary infections in women. J Fam Pract 23; 61-67, 1986.
2. Van der Meijden WI. Clinical aspects of *Gardnerella vaginalis*-associated vaginitis. A review of the literature. Scand J Urol Nephrol suppl 86: 135-141, 1985.

3. Burmeister RE, Gardner HL. Vaginitis: diagnosis and treatment. Postgrad Med 48: 159-163, 1970.
4. Stanley VC, Jones JB, Hurley R, Foulkes JEB, De Louvois J. Morbidity of the lower genital tract during pregnancy. J Clin Path 28: 736-740, 1975.
5. Fleury FJ. Adult vaginitis. Clin Obstet Gynecol 24: 407-438, 1981.
6. Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 141: 137-143, 1980.
7. Bergman JJ, Berg AO, Schneeweiss R, Heidrich FE. Clinical comparison of microscopic and culture techniques in the diagnosis of *Candida* vaginitis. J Fam Pract 18: 549-552, 1984.
8. Spence MR, Hollander DH, Smith J, McCaig L, Sewell D, Brockman M. The clinical and laboratory diagnosis of *Trichomonas vaginalis* infection. Sex Transm Dis 7: 168-171, 1980.
9. Chen KCS, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. J Clin Invest 63: 828-835, 1979.
10. Norusis MJ. SPSS/PC+. Advanced statistics for the IBM PC/XT/AT. SPSS inc., Chicago, 1986.
11. Lachenbruch PA. Discriminant analysis. Hafner Press, New York, 1975.
12. Bell TA, Farrow JA, Stamm WE, Critchlow CW, Holmes KK. Sexually transmitted diseases in females in a juvenile detention center. Sex Transm Dis 12: 140-144, 1985.
13. Hill LH, Ruparelia H, Embil JA,. Nonspecific vaginitis and other genital infections in three clinic populations. Sex Transm Dis 10: 114-118, 1983.
14. Kinghorn GR, Waugh MA. Oral contraceptive use and prevalence of infection with *Chlamydia trachomatis* in women. Br J Vener Dis 57: 187-190, 1981.
15. Staerfelt F, Gundersen TJ, Halsos AM, Barlinn C, Johansen AG, Nørregaard KM, Eng J. A survey of genital infections in patients attending a clinic for sexually transmitted diseases. Scand J Infect Dis suppl 40: 53-57, 1983.
16. Rothenberg RB, Simon R, Clipperfield E, Catterall RD. Efficacy of selected diagnostic tests for sexually transmitted diseases. JAMA 235: 49-51, 1976.
17. Gardner HL, Dukes CD. Clinical and laboratory effects of metronidazole. Am J Obstet Gynecol 89: 990-995, 1964.
18. Catterall RD. Diagnosis of vaginal discharge. Br J Vener Dis 46: 122-123, 1970.
19. Bergman JJ, Berg AO. How useful are symptoms in the diagnosis of *Candida* vaginitis? J Fam Pract 16: 509-511, 1983.
20. McCue JD, Komaroff AL, Pass TM, Cohen AB, Friedland G. Strategies for diagnosing vaginitis. J Fam Pract 9: 395-402, 1979.

IV CLINICAL AND LABORATORY FINDINGS IN WOMEN WITH BACTERIAL VAGINOSIS AND TRICHOMONIASIS VERSUS CONTROLS*

Willem I. van der Meijden[1], Hugo J. Duivenvoorden[2], Hedi C. Both-Patoir[3], Martine E. Hazen-Engelsman, and Aat C. Drogendijk[4]

Summary

We report comprehensively on the clinical and laboratory findings in 30 women with bacterial vaginosis(BV), 30 with vaginal trichomoniasis and 30 with normal secretions. Women with trichomoniasis were more often divorced (X^2 test, $p<<0.001$), more often complained of dyspareunia (X^2 test, $p<0.05$), frequently had discharge present in the vaginal vestibule, and showed one or more signs of vaginitis in half the cases. A 'moth-eaten' cervix was seen in only four women (13%) with trichomoniasis, but can be considered pathognonomic of the condition. While the main characteristics of vaginal secretions, i.e. amount, consistency, colour, absence or presence of gas and odour, are only poorly discriminative between BV and trichomoniasis, they can be of much help in distinguishing between abnormal and - probably - normal secretions. Parabasal cells were found in the wet mount of 19 women (73%) with trichomoniasis. Epithelial cell clusters were a prominent finding in controls but were virtually absent in the other two groups. We detected curved rods in 15 women (50%) with BV, but in none of

* Submitted for publication (Eur J Obstet Gynecol Reprod Biol)

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands
[2] Department of Psychotherapy and Medical Psychology, Erasmus University, Rotterdam, The Netherlands
[3] Department of Medical Microbiology, Erasmus University, Rotterdam, The Netherlands
[4] Department of Obstetrics and Gynaecology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands

the women with trichomoniasis or normal secretions. There were only minor differences in the results of bacterial cultures in case of BV and trichomoniasis. *Bacteroides* and *Peptostreptococcus* species were the predominant anaerobes in both groups. *Gardnerella vaginalis* was significantly more often isolated from women with BV than from controls (X^2 test for trend, $p<0.001$). Gas-liquid chromatography showed the presence of succinate in only two cases of BV. Lactate was found in all controls but one. Ethylation of vaginal samples probably reduces the risk of over-emphasizing the presence of succinate and lactate. It is concluded that BV and trichomoniasis are both characterized by the overgrowth of anaerobic bacteria and that there is a considerable overlap in clinical and laboratory findings. Microscopy of the wet mount should be considered the most powerful diagnostic tool.

Introduction

As shown in a recent survey in general practices, troublesome vaginal discharge is a frequent complaint, especially among younger women[1]. Although most types of vaginitis are primarily a threat to the comfort rather than to the health of the individual, most vaginal infections cause physical and sometimes also emotional distress. Women seeking help, whether consulting their general practitioner or referred to gynaecologist or dermatovenereologist, are therefore entitled to skilful investigation. The idea that an adequate diagnostic approach is the basis of effective treatment seems generally accepted.

The great majority of cases of abnormal discharge are related to specific disease entities, i.e. vaginal candidosis, trichomoniasis and bacterial vaginosis (BV). The latter condition, formerly named non-specific vaginitis, is the most prevalent [2]. Many articles have been published concerning the different clinical syndromes and the optimal way of establishing the diagnosis. Much recent research has focused on diagnosis and treatment of BV [3,4], while publications on trichomoniasis mainly concerned treatment regimens [5,6]. It is obvious that there still is a wide range of diagnostic attitudes, varying from the absolute minimum (let's have a look) to the use of sophisticated bacteriological methods (e.g. gas-liquid chromatography). The extent of the diagnostic efforts surely depends from severity and duration of complaints as well as on the technical facilities (general practice vs. hospital equipment). The physician's interest in problems of this kind is probably also important.

While BV and trichomoniasis undoubtedly have much in common, both

being characterized by malodorous, homogeneous and sometimes frothy discharge, the question is whether the difference between the two entities ends with the pathognomonic presence of clue cells or trichomonads, or whether other discriminating features can be detected. In order to establish this we performed an extensive clinical and laboratory study of 30 patients with BV and 30 with trichomoniasis, the latter not showing clue cells in the wet mount. Thirty women with normal vaginal secretions served as controls.

Material and Methods

Study population. Patients and controls were selected from women of childbearing age visiting the out-patient department of gynaecology for whatever reason. Women who had used antibiotics within the preceding month were excluded. The last sexual intercourse should not have taken place within 72 hours. Women with BV or trichomoniasis should not use oral contraceptives, while controls should not use contraceptives at all. The latter were only included if they had regular periods.
Anamnestic data. At the first visit some demographic data as well as those concerning the gynaecological history were recorded, with special emphasis on complaints related to vaginal discharge.
Gynaecological examination and specimen collection. This was always done by the same investigator (vdM). The external genitalia were examined, especially for signs of vulvitis and for the presence of discharge in the vaginal vestibule. After insertion of an unlubricated bi-valve speculum the gross aspects of cervix and vagina were noted, as well as the main characteristics of the vaginal discharge. The vaginal pH was measured half-way down the right lateral vaginal wall by means of a glass-calomel electrode with an accuracy of 0.02 pH (Electrofact, Amersfoort, The Netherlands). Samples for bacterial cultures were collected by rotating cottonwool swabs over the right lateral vaginal wall, after which they were placed in Stuart's transport medium until further processing, which generally took place within 1 to 3 hours. In order to avoid mixture of cervical mucus and vaginal secretions, all the visible mucus was removed with a piston pipette (Capilettor, Labora Mannheim, FRG). All the visible secretions (rotating of speculum) were then removed and collected in a pre-weighed autoanalysis cup (1.5 ml). This was done with a specially developed device (fig.1). The vaginal samples were weighed by means of an electronic top-loader (Sartorius, Göttingen, FRG), model 1219 MP, with an accuracy of 0.01 gramme. Cervical cytology was performed in all women, with a modified Ayre spatula. The presence of malodour was determined by smelling the secretions present on the withdrawn speculum.

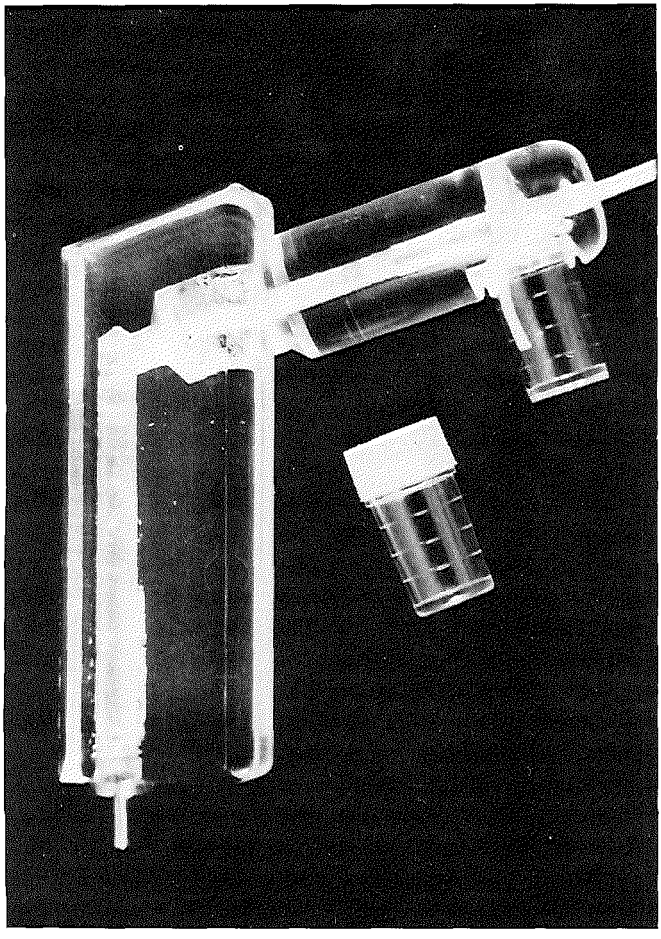


Fig. 1. Suction device (connected to water jet vacuumpump) Magnification 0.85x
Modified from Hage[18].

Some KOH 10% was then dripped onto the lower valve of the speculum and mixed with the discharge; when a rotten fish odour arose, the amine test was recorded as positive.

Microscopy. The wet mount was prepared by thoroughly mixing of a loopful of discharge with a loopful of normal saline. Microscopic fields were examined at a magnification of 400x. The presence of clue cells, bacterial

lumps, pseudo-hyphae, trichomonads, parabasal cells and leucocytes was especially noted. Vaginal secretions were also Gram-stained and studied in oil immersion (1000x), focusing on the overall impression of the bacterial flora, i.e. mainly Gram-positive, Gram-negative or 'miscellaneous'. The remaining vaginal secretions (autoanalysis cup) were deep-frozen (-20°C) for gas-chromatographic analysis on fatty acids in due time.

Clinical diagnoses. BV was diagnosed when the vaginal discharge fulfilled at least 2 of the following criteria: homogeneous consistency ('pasty'), pH<4.5, positive amine test and clue cells present [3]. The discharge was considered normal when none or only one of the BV criteria was met. The diagnosis 'vaginal trichomoniasis' depended on the presence of moving trichomonads in the wet mount. The presence of clue cells at the same time was a reason for exclusion, as was the presence of pseudo-hyphae in either of the three categories.

Bacterial cultures. Specimens for isolation of *Neisseria gonorrhoeae* were incubated on modified Thayer-Martin medium in an atmosphere of 10% CO_2, for 2 days at 37°C. Vaginal aerobes were isolated on blood agar. The plates were incubated for 2 days at 37°C. Candidal cultures were inoculated onto Sabouraud's agar and incubated for 3 days at 22°C. Anaerobes and *Gardnerella vaginalis* were isolated and identified as described elsewhere [7].

Gas-liquid chromatography. Specimens were prepared by a method modified from Carlsson [8]. Samples were acidified by addition of 0.1 ml of a 50% aqueous solution of H_2SO_4, after which 1 ml ethanol was added. Ethanol, fatty acids and ethyl esters were separated and analysed with a Packard-Becker model 428 gas-chromatograph equipped with hydrogen flame ionization detectors and a Hewlett-Packard integrator model 3380 A. Nitrogen was used as carrier gas. The glass column (1.8 mm by 4 mm inner diameter) was packed with chromosorb 101, 80-100 mesh (Chromopack Nederland B.V., Middelburg, The Netherlands). The operating temperatures were: inlet 210°C, detector 240°C, oven 220°C (ethanol and volatile acids) and 240°C (non-volatile acids). The gas flow rates were: nitrogen 40 ml/min, hydrogen 40 ml/min, and air 400 ml/min. The relative retention times were determined with reference standards of ethanol, acetic, propionic, isobutyric, n-butyric, isovaleric and n-valeric acids 2.5 mmol/l and lactic and succinic acids 2.0 mmol/l. The minimum detection level was 2.0 mmol/l.

Treatment. Women with BV were enrolled in a double-blind, placebo-controlled trial with a single dose (2 grammes) of tinidazole[7]. Vaginal trichomoniasis was also treated with 2 grammes of tinidazole. In the case of (partial) treatment failure we prescribed metronidazole, 4x250 mg for one

week. Steady partners were treated in the same way. Here we only report data on the first visit (intake).
Statistical methods. In the case of nominal and semi-quantitative variables, overall significance was tested by the X^2 test. Yates' correction was applied in the case of 2x2 tables. In order to compare pairwise differences in the groups studied, the X^2 test for trend was used[9]. Differences in continuous variables were tested by means of the Kruskal-Wallis test.

Results

Demographic data on the study population are listed in table I. Women with vaginal trichomoniasis were divorced significantly more often than women with BV or those with normal secretions ($p<<0.001$).
Women with trichomoniasis did not use contraception in half the cases. Nine women (32%) had an irregular menstrual period, versus only two women with BV. Sexual intercourse was described as 'casual' by 11 women (37%) with a *T. vaginalis* infection, while it was more often 'regular' in the other two groups.

Table I. Demographic data on the study population

	BV (n=30)	trichomoniasis (n=30)	controls (n=30)
Mean age ± SD	33.7 ± 7	34.3 ± 9.5	33 ± 5.6
Marital status			
married	19(63)	11(37)	27(90)
living together	8(27)	6(20)	2(7)
single	3(10)	6(20)	1(3)
divorced	0(0)	7(23)	0(0)
Contraception			
IUD	8(27)	3(10)	0(0)
female sterilization	16(53)	11(37)	26(87)
male sterilization	4(13)	1(3)	3(10)
none	2(7)	15(50)	1(3)

( ) number in parentheses = percentage

Table II. Vaginal and vulval complaints in women with BV or trichomoniasis, and in controls

	BV (n=30)	trichomoniasis (n=30)	controls (n=30)	p-value*
profuse discharge	21(70)	22(73)	16(53)	NS
malodorous discharge	24(80)	18(60)	5(17)	<<0.001
pruritus	3(10)	8(27)	7(23)	NS
irritation	5(17)	5(17)	7(23)	NS
dyspareunia	1(3)	6(20)	1(3)	<0.05
no complaints	4(13)	6(20)	14(47)	<0.01

( ) number in parentheses = percentage
* X^2 test
NS = not significant

As shown in table II, controls had specific complaints in about half the cases, profuse discharge being the major problem. The great majority of women with BV or trichomoniasis had one or more complaints, profuse and malodorous discharge being most important. Women with trichomoniasis more often complained of dyspareunia than women with BV or controls.
Most of the clinical findings are shown in table III. Vulvitis was noted in only one patient (trichomoniasis). Discharge was significantly more often present in the vaginal vestibule of women with trichomoniasis than in that of women with BV or controls. The introduction of the speculum caused discomfort in 12 women (40%) with trichomoniasis versus only two women with BV and one woman with normal secretions. Vaginitis, defined as the presence of a red vaginal mucosa with or without swelling and petechiae, was a prominent finding in women with *T. vaginalis* (50%). A 'moth-eaten' cervix was seen in four women (13%) with trichomoniasis. It was not present in women with BV or normal secretions.
As far as the amount of discharge is concerned, we found a significant difference only between trichomoniasis and controls, discharge being more often scant in the latter. While floccular, pasty (homogeneous) or watery secretions were a frequent finding in controls, BV and trichomoniasis respectively, curdy secretions were not seen in any patient. Discharge was white or whitish-yellow in the majority of women with BV and controls. In the case of trichomoniasis, however, discharge was more often yellowish. Four women (14%) from this group had blood-tinged discharge. A frothy discharge was noted in 14 women (47%) with BV and in 18 women (60%) with trichomoniasis. This difference is not statistically significant. Normal secretions were always non-odorous, i.e. *not* malodorous. While offensive

Table III. Clinical findings in women with BV or trichomoniasis, and in controls

	BV (n=30)	trichomoniasis (n=30)	controls (n=30)	p-value*
Vulvitis	0(0)	1(3)	0(0)	NS
Discharge in vestibule	6(20)	17(57)	7(23)	<0.01
Discomfort on introduction of speculum	2(7)	12(40)	1(3)	<<0.001
Vaginitis	2(7)	15(50)	0(0)	<<0.001
Moth-eaten cervix	0(0)	4(13)	0(0)	NS
Amount of discharge				
scant	12(40)	9(30	20(67)	
moderate	15(50)	15(50)	10(33)	
profuse	3(10)	6(20)	0(0)	<0.05
Consistency				
floccular	1(3)	2(7)	28(93)	
curdy	0(0)	0(0)	0(0)	
pasty	22(73)	10(33)	1(3)	
watery	7(23)	18(60)	1(3)	<<0.001
Colour				
white	18(62)	3(10)	17(57)	
whitish-yellow	6(21)	10(34)	10(33)	
yellowish-white	2(7)	4(14)	3(10)	
yellowish-green	3(10)	8(28)	0(0)	
blood-stained	1(3)	4(14)	0(0)	<0.001
Frothiness	14(47)	18(60)	0(0)	<<0.001
Odour				
non-odorous	3(10)	5(17)	39(100)	
disagreeable	26(87)	20(67)	0(0)	
offensive	1(3)	5(17)	0(0)	<<0.001
Vaginal pH[a]	5.09±0.45	5.31±1.28	4.08±0.86	<<0.001≠
range	4.36-5.89	4.17-7.00	3.43-4.90	
Amine test				
negative	3(10)	9(30)	30(100)	
doubtful	5(17)	9(30)	0(0)	
positive	22(73)	12(40)	0(0)	<<0.001
Weight of discharge[b]	0.27±0.18	0.31±0.25	0.32±0.16	NS≠
range	0.05-0.79	0.05-1.12	0.06-0.70	

( ) number in parentheses = percentage
* X^2 test
≠ Kruskal-Wallis
NS = not significant
[a] mean value ± SD
[b] mean value ± SD, grammes

discharge was present in only one case of BV, it was noted in five women (17%) with trichomoniasis.

The mean vaginal pH of normal secretions was 4.08 ± 0.86. It was significantly lower than that of BV (5.09 ± 0.45) or trichomoniasis (5.31 ± 1.28). Although a trend seems present, the difference in pH between BV and trichomoniasis is not significant. The amine test was never positive in controls. It was positive in 73% of women with BV and 'doubtful' or negative in over half the women with trichomoniasis. There was no significant difference in mean weight between vaginal secretions collected from women in the different study groups.

Table IV shows the main results of microscopy. While there was no significant difference in the number of leucocytes between the three groups when tested 'overall', the X^2 test for trend showed a significant difference ($p<0.05$) between BV and controls, and trichomoniasis, the latter condition more often showing >30 leucocytes per high power field. Bacterial lumps,

Table IV. Main results of microscopy of wet mount and Gram-stained preparation from women with BV or trichomoniasis, and from controls

	BV# (n=30)	trichomoniasis (n=30)	controls (n=30)	p-value*
Wet mount				
Leucocytes				
0-10	11(38)	5(17)	14(48)	
10-20	4(14)	1(3)	1(3)	
20-30	2(7)	2(7)	1(3)	
>30	12(41)	22(73)	13(45)	NS
Bacterial lumps	30(100)	15(55)[c]	0(0)	<<0.001
Parabasal cells	5(17)	19(73)[d]	2(7)	<<0.001
Epithelial cell clusters	1(3)	2(7)[b]	26(96)	<<0.001
Gram-stain				
Bacterial flora				
mainly Gram + ve	0(0)	2(7) } [a]	30(100)	
mainly Gram – ve	26(87)	5(17) } [a]	0(0)	
'miscellaneous'	4(13)	22(76) } [a]	0(0)	<<0.001
Comma-shaped rods	15(50)	0(0)	0(0)	<<0.001

( ) number in parentheses = percentage

clue cells were present in 27 women with BV

* X^2 test

NS = not significant

[a-d] one-four missing value(s)

Table V. Bacterial species isolated from vaginal fluid from women with BV or trichomoniasis, and from controls

organism	BV (n=30)	Trichomoniasis (n=30)	Controls (n=30)
Aerobes/facultatives			
Escherichia coli	2	1	1
Enterobacter aerogenes	1	0	0
micrococci	1	2[1]	0
Neisseria gonorrhoeae	0	1	0
Candida albicans	1	1	1
Torulopsis glabrata	0	1	3(2)
Rhodotorula rubra	1	0	0
coagulase-ve staphylococci	12	10	5
coagulase+ve staphylococci	0	1	0
β-haemolytic streptococci			
Lancefield group B	4(1)	6(2)	0
α-haemolytic streptococci	3(2)	3(2)	1
non-haemolytic streptococci	1	6(4)	1
Streptococcus faecalis	1	0	2(1)
Gardnerella vaginalis	18(18)	11(11)	5(4)
Lactobacillus species	26(20)	23(17)	27(20)
average number of species	2.4	2.2	1.5
Obligate anaerobes			
Bacteroides melaninogenicus	10(6)	2(2)	0
Bacteroides corrodens	4(2)	0	0
Bacteroides fragilis	0	1(1)	0
Bacteroides species	16(12)	17(16)	0
Peptostreptococcus species	19(18)	14(14)	6(3)
Peptococcus species	0	3(2)	0
average number of species	1.6	1.2	0.2

number in parentheses = case(s) with moderate to heavy growth

aggregates of tiny, unmoving bacteria (presumably *G.vaginalis*), were seen in all women with BV, in 55% of women with trichomoniasis and in none of controls. Parabasal cells were significantly more often present in trichomoniasis than in BV or normal secretions (73% versus 17% and 7% respectively). Widely varying numbers of vaginal epithelial cells that were attached to one another (cell clusters) were a prominent finding in women with normal secretions, but were almost totally lacking in the other two

groups. The Gram-stained preparation was totally different in all three groups. Normal secretions showed the uniform picture of Gram-positive bacilli (of varying length), while in the case of BV the bacterial flora was most often Gram-negative and predominantly consisted of small bacilli. The 'miscellaneous' character of most Gram-preparations of trichomoniasis points to the presence of both Gram-positive and Gram-negative cocci and bacilli. Comma-shaped rods were detected in 50% of cases of BV. They were not present in trichomoniasis or normal secretions. *Leptothrix* was seen in two women with trichomoniasis.

Abnormal cervical cytology was found in four women. One woman with BV and two with trichomoniasis showed slight dysplasia, while another woman with BV had severe dysplasia. Signs of infection (large number of leucocytes and bacteria) were significantly more prevalent in smears from women with trichomoniasis than in smears from controls ($p<0.05$). Trichomonads were detected in 16 of 30 Papanicolaou smears from women with trichomoniasis (53%).

Bacteriological results are shown in table V. Among the aerobes and facultative anaerobes, coagulase-negative staphylococci, (non-) haemolytic streptococci, *G.vaginalis* and *Lactobacillus* species were the most frequently isolated bacteria. A significant difference in the isolation rate of *G.vaginalis* was found only between women with normal secretions and those with BV ($p<0.001$). Obligate anaerobes were almost exclusively found in BV and trichomoniasis. *Bacteroides melaninogenicus*, *Bacteroides* species and *Peptostreptococcus* species were the most prevalent. *B.melaninogenicus* was significantly more often isolated from women with BV than from those with trichomoniasis or controls (p-value <0.05 and <0.01 respectively). *B. fragilis* was isolated only once (trichomoniasis).

Gas-liquid chromatography (table VI) showed the presence of acetate in all women with BV (range 15-106 mmol/l), in 29 women with trichomoniasis (range 5-102 mmol/l) and in only nine controls (range 2-11 mmol/l). Propionate and butyrate were infrequently detected, only in women with BV. Succinate was present in only two women with BV(7%). The difference in detection rate between trichomoniasis and controls (23% versus 0%) is statistically significant. Lactate was found in one woman with BV (78 mmol/l). This woman had non-odorous discharge. It was present in four women with trichomoniasis (range 48-109 mmol/l). Three of these women were classified as 'asymptomatic carrier'. Lactate was found in 28 of 29 samples from controls (range 10-294 mmol/l).

Table VI. Volatile and non-volatile organic acids in vaginal fluid from women with BV or trichomoniasis, and from controls

	BV (n=30)	trichomoniasis (n=30)	controls (n=30)	p-value*
acetate	30(100)	29(97)	9(30)	<<0.001
propionate	3(10)	0(0)	0(0)	NS
butyrate	2(7)	0(0)	0(0)	NS
succinate	2(7)	7(23)	0(0)	<0.01
lactate	1(3)	4(13)	28(97)[a]	<<0.001

( ) number in parentheses = percentage

* X^2 test

[a] gas chromatography was not performed in one case

Discussion

Women with trichomoniasis were more often divorced than women from the other two groups. A high prevalence (37%) of trichomoniasis among divorced or separated women was also found in an epidemiological study in the USA[10]. This is perhaps explained by more casual relationships in this group. The finding that a considerable proportion of women with trichomoniasis did not use any form of contraception was commented on by other authors[11].

Temporary disturbances of the menstrual period are said to be a frequent finding in women with trichomoniasis[12]. However, studies of the role of gonadal hormones in this respect have produced contradictory data[13].

While women with BV or trichomoniasis had troublesome discharge significantly more often than controls, it is obvious that normal secretions also frequently give rise to complaints. The reasons for this are as yet unknown. It may be that 'the growing interest in personal health leads women to complain to their doctors of symptoms which in earlier age were borne in silence'[14]. Ample discussion with women about this subject may clarify the matter to some extent.

Our finding that the great majority of women with BV or trichomoniasis had vaginal or vulval complaints, seems to contradict data from other studies, which stress the total lack of symptoms in a considerable proportion of women with BV or trichomoniasis[15,16]. The method of patient selection is probably responsible, especially in the case of trichomoniasis, since we selected only women with a positive wet mount.

Spontaneous leucorrhoea, discharge present in the vaginal vestibule, was more often noted in women with trichomoniasis. This feature is certainly

correlated with the consistency of secretions, being thin in most cases of trichomoniasis, and thus easily running over the vulva. The fact that dyspareunia and pain upon introduction of the speculum were almost exclusively restricted to patients with trichomoniasis, is easily explained by the frequent finding of vaginitis in this group. Hoehne was the first to draw attention to the fact that *T.vaginalis* is a frequent cause of purulent vaginitis[17]. The 'moth-eaten' ('strawberry') cervix is generally found in less than 5% of cases of trichomoniasis[11,16], but can be considered pathognomonic of the condition.

The main characteristics of vaginal secretions, i.e. amount, consistency, colour and absence or presence of gas, do not seem to be of much help in discriminating between BV and trichomoniasis. This also applies to the absence or presence of vaginal malodour, the vaginal pH and the result of the amine test. However, some parameters, especially consistency and the absence or presence of vaginal malodour and frothiness, are very useful in identifying women with abnormal and those with - probably - normal discharge. The latter often present with floccular, non-odorous and non-frothy secretions. Since malodorous secretions always have an increased pH (>4.5), and since the pH of non-odorous secretions is mostly normal (≤ 4.5), the necessity of pH measurement as part of the diagnostic procedure is debatable.

There was no significant difference in mean weight of vaginal discharge collected from women with BV, trichomoniasis or controls, which of course does not mean that there could not have been significant differences in *volume* of discharge. The mean values in our study are in agreement with the results of a longitudinal study performed by Hage[18], but considerably lower than the 0.76-1 gramme reported by others[19].

More than 30 leucocytes per field (400x) were seen in 73% of women with trichomoniasis, but also in 41% of women with BV and in 45% of controls. Women with more than 10 white blood cells per high power field on a wet preparation, are supposed to be at an increased risk of infection by *T.vaginalis*[16]. The paucity of leucocytes in the wet mount is generally looked upon as a characteristic feature of BV, but our data do not support this. However, we did not investigate the possibility of concomitant cervical infection. Parabasal cells were frequently found in women with trichomoniasis, which is confirmed by others[20].

The inhomogeneity of normal secretions is probably based on the presence of epithelial cell clusters, which sometimes comprise hundreds of cells. The homogeneous discharge of BV and trichomoniasis contains these aggregates only very rarely.

The results of Gram-staining show that the character of the bacterial flora is rather different in BV, trichomoniasis, and controls. Other authors also demonstrated the usefulness of the Gram-stain in the diagnosis of abnormal discharge[21]. They distinguished between a *Lactobacillus* morphotype (large Gram-positive rods) and a *Gardnerella* morphotype (small Gram-variable rods). Gram-negative curved rods were detected in 44% of their patients with BV, which is consistent with our findings. We were unable to detect curved rods in any patient with trichomoniasis, the reason for which is unknown. The possibility of competitive growth inhibition should be investigated further.

Culture of vaginal secretions showed very little difference between women with BV and those with trichomoniasis. Although *G. vaginalis* was less often isolated from women with trichomoniasis, the difference is not significant. The finding of moderate to heavy growth of *G. vaginalis* in 11 women (37%) with trichomoniasis demonstrates that its presence per se does not necessarily lead to the formation of clue cells. Whether or not *B. melaninogenicus* preferably colonizes the vagina in the case of BV remains to be studied. In the literature data on the bacterial flora in vaginal trichomoniasis are scanty. It is evident, however, that obligate anaerobes, especially *Bacteroides* species and *Peptostreptococcus* species, are frequently isolated[22,23]. The bacterial flora in BV has been studied more extensively, especially during the last decade[24,25]. As in our study, *G. vaginalis* and obligate anaerobes were more often isolated from women with BV than from controls.

Our gas-chromatographic results differ from those reported by others[3,24,25], especially so far as the non-volatile acids (succinate, lactate) are concerned. While other investigators invariably make use of methanol in the preparation of specimens and of the S/L ratio (peak height succinate divided by peak height lactate) as an indicator of the presence or absence of BV, we ethylated our samples and determined the presence of succinate and lactate in a quantitative way (mmol/l). In only two cases of BV did we find succinate in a concentration above the minimum detection level (6 and 28 mmol/l respectively). Acetate was detected in all women with BV and in 29 women with trichomoniasis, versus in only nine controls. The concentrations were significantly lower in the latter (<20 mmol/l). We feel that our results are in accordance with the fact that, under optimal conditions, most obligate anaerobes that can be isolated from vaginal secretions in the case of BV or trichomoniasis mainly produce acetate, except for certain *Bacteroides* species which produce both succinate and acetate[26]. Ethylation of vaginal samples, producing lower peak heights, probably reduces the risk of over-

emphasizing the presence of succinate and lactate. A comparitive study (ethanol vs. methanol) is in progress.
While the combination of malodorous discharge and the presence of vaginitis and/or a 'moth-eaten' cervix is strongly indicative of trichomoniasis, it is evident that clinical parameters alone do not suffice for the distinction between BV and trichomoniasis. However, they deserve our attention because a preliminary diagnosis of 'normal' discharge can be reached easily.
The absence or presence of bacterial lumps, parabasal cells and cell clusters can help in preselection and in determining the need of cultures (*T. vaginalis, Candida* species). Despite the fact that the Gram-stained smear looks special in all three groups, its value as a diagnostic tool is limited. It is less suitable for recognition of clue cells and trichomonads than the wet mount. On the other hand, the detection of yeast (chlamydospores, pseudohyphae) is greatly enhanced because it is Gram-positive. It is also relatively easy to recognize curved rods. This could be of help in future research work.
Our study has shown that bacterial cultures and gas-liquid chromatography of vaginal secretions do not reveal important differences between BV and trichomoniasis. The culture of vaginal secretions should not (yet) be performed as a routine. It should, however, be reserved for cases in which a strong suspicion (anamnestic and/or clinical) of a specific vaginal infection is not supported by the result of wet mount microscopy. When one considers the possibility of gonococcal or chlamydial infection, the best place to take a culture is the uterine cervix. Gas chromatography is of help in the presumptive identification of anaerobes, but since it is within reach of only a few doctors, and given the fact that the diagnosis of an anaerobic vaginal infection is easily made by smelling the secretions, this technique has a place only in research work.
In conclusion, BV and trichomoniasis are vaginal disease entities both characterized by overgrowth of anaerobic bacteria. There is a considerable overlap in clinical and laboratory findings.
Microscopy of the wet mount still is the most powerful diagnostic tool.

References

1. Van der Meijden WI, Bosch I, De Haes WFM, Lako CJ, Van Harten RPW, Zorn H. Vaginale afscheiding: wat zeggen vrouwen er zelf over? Huisarts en Wetenschap 1985; 28:387-391.
2. Fleury FJ. Adult vaginitis. Clin Obstet Gynecol 1981; 24:407-438.
3. Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK. Anaerobic bacteria in nonspecific vaginitis. New Engl J Med 1980; 303:601-607.

4. Van der Meijden WI, Piot P, Schmitz PIM, Stolz E. Treatment of clue cell-positive discharge with 200 mg povidone-iodine pessaries. A double-blind and placebo-controlled trial. Eur J Obstet Gynecol Reprod Biol 1987; 24:299-307.
5. Fleury FJ, Van Bergen WS, Prentice RL, Russell JG, Singleton JA, Standard JV. Single dose of two grams of metronidazole for *Trichomonas vaginalis* infection. Am J Obstet Gynecol 1977; 128:320-322.
6. Lorenz K, Rüttgers H. Clinical experience using SolcoTrichovac in the treatment of trichomonas infections in women. Gynäk Rdsch 1983; 23, suppl 2:64-71.
7. Van der Meijden WI. Treatment of non-specific vaginitis with a single dose of tinidazole. Scand J Infect Dis 1983; suppl 40:85-89.
8. Carlsson J. Simplified gas chromatographic procedure for identification of bacterial metabolic products. Appl Microbiol 1973; 25:287-289.
9. Mantel N. Chi-square tests with one degree of freedom: extensions of the Mantel-Haenszel procedure. J Am Stat Assoc 1963; 58: 690-700.
10. Naguib SM, Comstock GW, Davis HJ. Epidemiologic study of trichomoniasis in normal women. Obstet Gynecol 1966; 27:607-616.
11. Fouts AC, Kraus SJ. *Trichomonas vaginalis*: Reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980; 141:137-143.
12. Holtorff J. Zur Problematik der sogenannten Trichomonaden Kolpitis. Deutsche Gesundheitswesen 1960; 15:1076-1083.
13. Birnbaum H, Kraussold E. Häufigkeit von Sprosspilz - und Trichomonadeninfektionen bei Anwendung hormonaler und intrauteriner Kontrazeption. Zbl Gynäkol 1975; 97:1636-1640.
14. Boycott JA, Oxon DM. The nature of vaginal discharge. Lancet *i*, 1961; 1071-1074.
15. Amsel R. Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983; 74:14-22.
16. McLellan R, Spence MR, Brockman M, Raffel L, Smith JL. The clinical diagnosis of trichomoniasis. Obstet Gynecol 1982; 60:30-34.
17. Hoehne O. *Trichomonas vaginalis* als häufiger Erreger einer typischen Colpitis purulenta. Zbl Gynäkol 1916; 40:4-15.
18. Hage JC. Over het ontstaan van het zure milieu in de vagina. Thesis, 1978.
19. Stone A, Gamble CJ. The quantity of vaginal fluid. Am J Obstet Gynecol 1959; 78:279-281.
20. Koss LG, Wolinska WH. *Trichomonas vaginalis* cervicitis and its relationship to cervical cancer. Cancer 1959; 12:1171-1184.
21. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. J Clin Microbiol 1983; 18:170-177.
22. Hite KE, Hesseltine HC, Goldstein L. A study of the bacterial flora of the normal and pathologic vagina and uterus. Am J Obstet Gynecol 1947; 53:233-240.
23. Brockmann J, Höhne C. Bakteriologische Aspekte der Trichomonadenkolpitis. Zbl Gynäkol 1979; 101:722-726.
24. Piot P, Van Dijck E, Godts P, Vanderheijden J. The vaginal microbial flora in non-specific vaginitis. Eur J Clin Microbiol 1982; 1:301-306.
25. Embree J, Caliando JJ, McCormack WM. Nonspecific vaginitis among women attending a sexually transmitted diseases clinic. Sex Transm Dis 1984; 11:81-84.
26. Holdeman LV, Cato EP, Moore WEC. Anaerobe Laboratory Manual, 4th ed, VPI Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Va., 1977.

V THE EXPERIENCE OF TROUBLESOME VAGINAL DISCHARGE. RESULTS OF A SURVEY AMONG 5,900 WOMEN IN GENERAL PRACTICES*

Willem I. van der Meijden[1], Willy F.M. de Haes[2], Ina Bosch[3], Christiaan J. Lako[3], Robert P.W. van Harten[4], Hans Zorn[4]

Summary

In order to get an idea of the prevalence of troublesome vaginal discharge and to gain insight into factors associated with it, a survey was carried out among 5,900 women. The questionnaires for this survey were distributed by general practitioners and were returned by 3,168 women (54 % response). Troublesome vaginal discharge was experienced by 43 % of the respondents. The complaint was strongly related to the age of the respondents, and decreased by about 10 % per 10-year period ($p<0.001$). There was no significant relation between troublesome discharge and the use of tampons, the use of certain contraceptives and sexual intercourse. Women with a history of treatment for vaginal discharge generally went to see the doctor with specific complaints. Fear of venereal disease was not an important motive, except -relatively- in age group 15-24 years.
Information on vaginal discharge was considered desirable by 61 % of the respondents, and should preferably be supplied by general practitioners, women's magazines and television.

* Submitted for publication (Patient Educ Couns)

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands
[2] Municipal Health Department, Health Education Unit, Rotterdam, The Netherlands
[3] Department of Environmental and Tropical Health, Agricultural University, Wageningen, The Netherlands
[4] Rhône-Poulenc Nederland B.V., Amstelveen, The Netherlands

Introduction

Troublesome vaginal discharge is a problem with which many women, especially the sexually mature, are confronted. It has been estimated that between five and 10 % of women seen in general practice complain of vaginal discharge. A considerable proportion of the patients seen in gynaecological and contraconceptive clinics, as well as in departments of venereology, present with this symptom[1]. Approximately one-third of women of childbearing age would currently have one or more types of vaginitis[2].

The prevalence of abnormal vaginal discharge among the users of oral contraceptives and IUD is said to be higher than that among non-users[3,4]. The more or less continuous use of tampons could also cause trouble[5]. It is generally agreed that promiscuous women have a greater risk for vaginal infections, than women with a steady sexual relationship.

The available information on the extent of the problem of vaginal discharge is incomplete. Hard figures, which propably represent only the tip of the iceberg, are being collected by several sentinel stations. Data provided by the Monitoring Project show that vaginitis and vulvitis are among the most common diseases of the female urogenital system. These diagnoses are made mostly in women aged 15-44 years[6]. Morbidity data from the Nijmegen University Institute of General Practitioners show that a general practitioner in a so-called standard practice (2,800 registered patients) is likely to be confronted with about 72 new cases of 'vaginitis' per year[7]. In addition it is possible to gain a (rough) impression of the incidence of abnormal vaginal discharge from the number of prescriptions issued for this indication. It was calculated from data supplied by the Institute for Medical Statistics (IMS) in The Hague that the diagnosis 'vaginal infection' (which includes trichomoniasis, candidosis, vaginitis, vulvitis, etc.) is annually made about 525,000 times in women aged 12-54 years (n=4.3 million).

The true incidence is probably much higher. Perhaps shame prevents many women from presenting with the complaint 'vaginal discharge'. When the practitioner belittles the complaint or fails to make the diagnosis for whatever reason, this may of course also lead to significant underreporting. Data on views of women on vaginal discharge and their behaviour in this context are virtually lacking. Do women regard vaginal discharge as ' something one has to accept' or do they tend to take this problem to the physician? How many women regard vaginal discharge as troublesome? Are women well-informed on the subject and, if not, how should in their opinion the information be given? In order to find answers to these questions a survey was held among 5,900 women in 1983.

Method

Questionnaire. In preparing the questionnaire efforts were made to keep the questions as simple as possible, avoid medical terminology and limit the number of questions so as to achieve an optimal response. Moreover, the questionnaire was accompanied by a letter explaining why the enquiry was held and what might be done with the results.

In terms of substance the questions can be divided as follows:

- general data (age and occupation);
- factors which might influence vaginal discharge (menstrual hygiene, use of contraceptives, sexual activity/ promiscuity);
- reaction to vaginal discharge;
- occurrence of troublesome discharge (past or present);
- need for information and preferred methods

With most questions a limited number of possible answers were indicated; only in some instances was the possibility 'other, namely ....' given. At the end of the questionnaire, moreover, a space was reserved for remarks. The questionnaire was accompanied by a post-free return envelope as well as the explanatory letter.

Pilot study. A pilot study was first performed in two general practices in Arnhem. The aim was to test the issuing procedure, to establish whether the questionnaire served its purpose, and to gain some impression of the response. The results of this pilot study were such as to prompt the decision to use the same procedure and questionnaire in a nationwide study.

Recruiting of the study population. In the context of a refresher project (diagnosis and therapy of vaginitis) one of the authors (vdM) attended meetings of general practitioners at which he asked those present to participate in the study. This participation consisted of the issuing of the questionnaire to one-hundred consecutive women aged 15-75 who attended surgery hours. These women were to complete the questionnaire unaided. A total of 64 general practitioners, four of them in each of 16 different Dutch communities (figure 1), promised to cooperate so that, in principle, 6,400 questionnaires could be issued.

Statistics. For statistical analysis the Statistical Package for the Social Sciences (SPSS) was used[8]. The significance of relations was tested by means of the X^2 test. In order to make a correction for the effects of confounding variables we used the method of direct standardization[9].

Figure 1. Sites in The Netherlands covered by the enquiry.

■ city (urban residential centre, population 100,000 and over)

□ town (residential centre, population 10,000 - 29,999)

● commuter community (male working population includes over 30 % mainly allochthonous resident commuters)

○ urbanized rural community (population 5,000 - 29,999)

▲ rural community (20 - 39,9 % agrarian working population)

(criteria applied by Central Bureau of Statistics)

Results

Response. Some general practitioners failed to distribute all questionnaires so that in the end not the maximum number but 'only' 5,900 questionnaires were issued (92 %). The questionnaires were returned by 3,168 women (54 %), and most of these questionnaires had been fully completed.
The mean age of the respondents was 33 years (figure 2). Two-thirds of these women listed 'housewife' as occupation.

1. This study
2. Maandbericht Gezondheisstatistiek (CBS), 83/10
3. Central Bureau of Statistics, (census date 1st January, 1982).

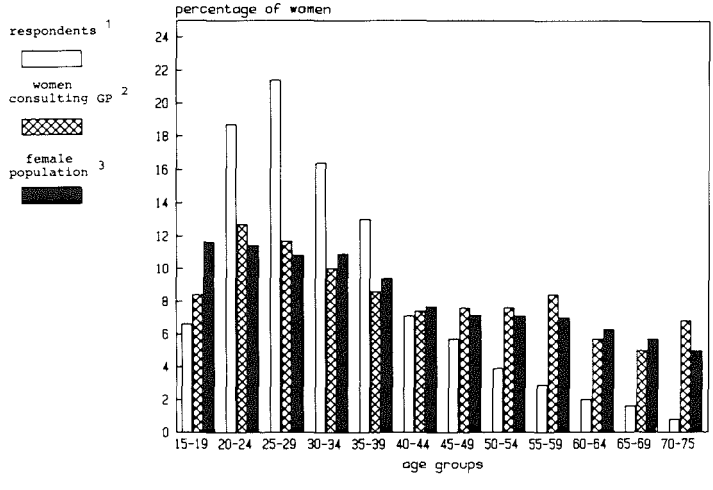


Figure 2. Age distribution of the respondents and two reference groups

Prevalence of troublesome vaginal discharge. Troublesome discharge was reported by 42% of the respondents. The complaint was strongly related to the age of the respondents: the percentage of women with troublesome discharge decreased by about 10% per 10-year period. After standardization for a number of variables, the relation between age and troublesome discharge persisted (table 1).
A significant relation was also found between troublesome discharge and

Table 1. Percentage of women having troublesome discharge, in different age groups and standardized for different variables

	15-24 (n=756)	25-34 (n=1111)	35-44 (n=585)	45-54 (n=264)	55-75 (n=181)
Not standardized	57	48	36	27	17
Standardized for					
Menstrual hygiene	58	49	35	30	–*
Contraceptive method	60	49	29	25	–*
Having/not having multiple partners	57	48	37	27	19
Having/not having 'regular' sexual intercourse	57	48	35	27	16

* some percentages have been discarded, the number of cases being too small

treatment for this complaint in the past ($p<0.001$). Previous treatment for vaginal discharge was reported by 45% of the respondents. The percentages in the various age groups were:

- 15-24 years: 39 %
- 25-34 years: 57 %
- 35-44 years: 48 %
- 44-54 years: 41 %
- 55-75 years: 28 %

Table 2 shows that women previously treated for vaginal discharge generally had specific complaints. Discharge, itching and disagreeable odour were the

Table 2. Reasons for encounter as indicated by women who had been treated for vaginal discharge. Percentages per age group

	15-24 (n=75)	25-34 (n=290)	35-44 (n=592)	45-54 (n=287)	55-75 (n=120)
Troublesome discharge	66	67	50	53	43
Odour	38	31	32	34	29
Itching	57	63	69	63	44
Painful intercourse	19	18	15	13	13
Fear of VD	10	3	3	1	7
Fear of cancer	2	6	10	6	11
Other	10	11	12	10	13

principal reasons for encounter. Fear of venereal disease was not an important reason except - relatively - in age group 15-24 years.

Possibly predisposing factors. Table 3 lists a number of factors which might play a predisposing role in causing (troublesome) vaginal discharge. Tampons were used mostly in age group 15-24 years. In later age groups the popularity of tampons rapidly diminished; sanitary pads were generally preferred. Oral contraceptives were used mostly by women younger than 35 years, and more especially in age group 15-24 years. The IUD does not seem to be a very popular contraceptive method. Especially women in age group

Table 3. Presence of possibly predisposing factors. Percentages per age group

	15-24	25-34	35-44	45-54	55-75
Menstrual hygiene	(n=759)	(n=1125)	(n=598)	(n=283)	(n=213)
Sanitary pads	38	54	61	44	1
Tampons	30	14	7	1	–
Alternately pads/tampons	31	30	23	11	1
No menstruation(any longer)	1	2	9	44	98
Contraception	(n=758)	(n=1123)	(n=590)	(n=272)	(n=157)
Pill	69	43	18	8	1
IUD	4	15	6	3	–
Condom	5	14	8	11	1
Female sterilization	–	5	28	20	1
Male sterilization	1	10	22	15	2
Other methods	4	8	6	12	8
No contraception	20	16	24	43	83
Sexual activity	(n=757)	(n=1127)	(n=598)	(n=282)	(n=207)
No regular sexual contact	17	6	9	16	29
Regular sexual contact	74	92	90	78	46
No sexual contact	9	2	1	6	26
Sexual behaviour	(n=751)	(n=1122)	(n=594)	(n=273)	(n=180)
No sexual partner	17	4	5	10	36
One steady sexual partner	78	94	93	89	63
Several steady partners	1	1	1	–	1
Varying sexual partners	3	1	1	1	1

Table 4. Percentage of women having troublesome discharge, using certain menstrual hygienic (top) and contraceptive (bottom) methods. Data before and after standardization for age

	sanitary pads (n=1417)	tampons or tampons/pads (n=1218)	no menstruation (n=416)
Not standardized	45	49	23
Standardized	48	48	51

	barrier methods (n=314)	IUD (n=236)	oral contraceptives (n=1125)	other non-barrier methods (n=148)
Not standardized	47	51	50	35
Standardized	42	45	40	35

15-24 years rather infrequently used the IUD. Definitive contraception (male or female sterilization) played a prominent role in age group 35-44 (50%). Other contraceptive methods (e.g. spermicide, coitus interruptus, periodic abstinence) were used in particular by elder women. The most regular sexual contacts were reported by women aged 25-44 years. The largest percentage of varying sexual partners was found in age group 15-24 years. After standardization for age, no significant relation was found between the use of tampons or certain contraceptives on the one hand, and troublesome discharge on the other (table 4). Nor was a significant relation demonstrable between regular sexual contacts or varying sexual partners on the one hand, and troublesome discharge on the other ($p<0.1$ and $p<0.5$ respectively).

Table 5. Reactions to vaginal discharge. Percentage per age group*

	15-24 (n=751)	25-34 (n=1108)	35-44 (n=582)	45-54 (n=270)	55-75 (n=184)
Something normal	47	41	38	44	26
Expectant attitude	20	18	17	18	8
Panty liners	31	31	21	12	9
Water and soap	9	10	12	14	19
Water	29	30	27	23	24
General practitioner	25	33	38	32	42
Other	7	8	5	4	3

* Total of percentages can be over 100, because more than 1 answer is possible

Table 6. Need for information about vaginal discharge. Percentages per age group

	15-24 (n=751)	25-34 (n=1109)	35-44 (n=576)	45-54 (n=264)	55-75 (n=171)
Needs information	75	67	59	45	30
Knows enough	19	25	31	36	37
Not interested	2	2	3	7	11
No, because	4	6	7	13	23

Reactions to vaginal discharge. Many women regarded vaginal discharge as 'something normal' (table 5), but about one-third of the respondents indicated they would see a doctor about it. An expectant attitude was adopted mostly by women younger than 55 years.

Need for information and preferred methods. The need for information about vaginal discharge in the different age groups is shown in table 6. Sixty-four % of the respondents wanted information on the subject, the percentages in the different age groups showing a maximum in age group 15-24 years (75%), steadily decreasing thereafter to 30% in age group 55-75 years ($p<0.001$). Women having troublesome discharge had a significantly greater need for information than women who did not have troublesome discharge ($p<0.001$). Women who reported having no need for information for other reasons than 'know enough' or 'not interested' usually indicated that they had no vaginal discharge. All age groups agreed that information should be preferably given by the general practitioner (figure 3). Women's magazines and television were also considered important. An alternative of some importance would seem to be a folder for patients which should be available (preferably free of charge) from general practitioners and dispensing chemists (waiting rooms).

Remarks. The space left for remarks on the questionnaire was used by 899 women (28%). The remarks often concerned personal experience with vaginal discharge and the information on the subject.

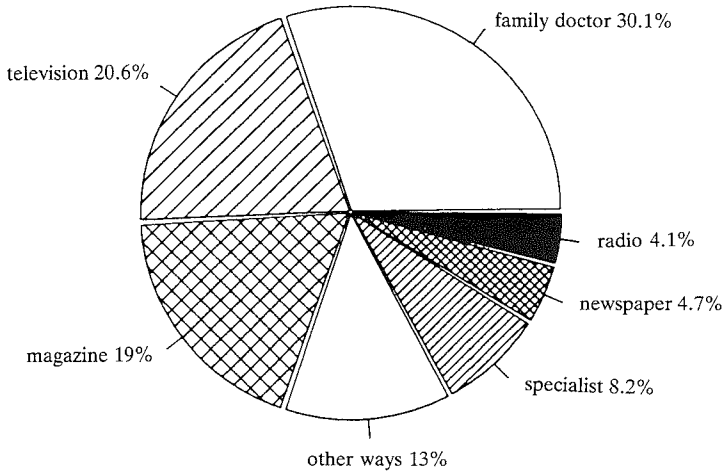


Figure 3. Ways of getting information as preferred by the respondents

Discussion

It might be concluded from the results of this study that the number of women bothered by vaginal discharge is much larger than the number of women who in the end take this complaint to a doctor; however, this conclusion invites some comment.

The investigators proceeded from the postulate that a positive answer to the question 'were you bothered by vaginal discharge...?' in fact implied troublesome discharge. However, some women may only have indicated the presence of vaginal discharge with this answer, without experiencing it as really troublesome.

In this study no attempt was made to test the women's subjective findings (trouble/no trouble) against the physician's findings (abnormal/normal discharge). This would have been impossible in a study of this scope. Moreover, the opinions of women and the findings of physicians in this respect are not infrequently discrepant[10,11]. It is quite conceivable, for instance, that normal (physiological) vaginal discharge is experienced as troublesome, and the reverse is equally true.

Figure 2 shows that the age distribution of all women in The Netherlands does not differ much from that of all women who visit a general practitioner, but the age distribution of the respondents differs significantly in that younger women are markedly overrepresented. There may be three main reasons for this:

- the general practices chosen were not representative;
- the participating general practitioners were selective in issuing the questionnaires (especially to women with vaginal discharge);
- relatively many older women did not return the questionnaire

It is impossible exactly to indicate the factor which has played the principal role, although it seems justifiable to assume that the first two have been of minor importance. It is a plausible assumption that women with troublesome discharge, i.e. mostly younger women (table 1), were more inclined to return the questionnaire than women not bothered by vaginal discharge. This is why the percentage of women with troublesome vaginal discharge would probably be smaller in a more representative sample.

As already mentioned in the introduction, the 'hard' data on vaginal discharge (from sentinel stations and IMS) may represent only the tip of the iceberg. It is probably true that not all women with troublesome discharge consult a doctor about it. They may for instance believe that it is 'just one of those things'.

The fact that only a small percentage of women who had been treated for vaginal discharge suspected a venereal disease (table 2), could indicate that not many women are aware of the possibility that troublesome discharge is one of the (first) signs of a venereal disease.

Embarrassment or shame may lead to postponement behaviour, and perhaps especially in older women. In a recent doctoral thesis it was concluded that women who present with vaginal discharge as complaint show more postponement behaviour and rigidity than women attending surgery hours with other gynaecological complaints[12].

The attitude of the physician to whom a woman addresses the complaint of vaginal discharge is of course very important. Belittling the complaint may lead to underreporting; and insufficient knowledge of the diagnostic possibilities may also contribute to this. Both factors should be taken into account.

No significant relation was demonstrated between the use of tampons or certain contraceptives and troublesome discharge (table 4), even though the former are in some studies mentioned as predisposing factors[3-5]. The previously mentioned discrepancy between the subjective findings of the patients and the findings of the physician may be involved in this respect.

Having several/varying sexual partners did not seem to be of any influence. It is to be noted, however, that the numbers in both groups were very small (table 3).

Our study reveals a great need for information, which is expressed by 75% of the younger patients. However, 30% of the women older than 55 years also ask for more information. Women troubled by vaginal discharge state their need for information more frequently than women not troubled by it (75% versus 53%); the latter percentage, however, is still remarkebly large. The findings warrant the conclusion that no group is to be excluded a priori from information.

What should information comprise? Research data show that many women regard vaginal discharge as 'something normal'. Information should therefore focus primarily on such questions as 'what is normal vaginal discharge?' and 'when should I seek medical advice about vaginal discharge?' It should be pointed out to women that the cause of the inconvenience can usually be traced by a simple examination.

Moreover, information should concern possibilities of treating vaginal discharge, paying ample attention also to alternative methods of treatment. Since general practitioners are sometimes insufficiently familiar with the diagnostic and therapeutic possibilities in relation to vaginal discharge (Van der Meijden, personal communication), refresher courses on this subject should be provided. If not, then women who present with troublesome vaginal discharge in general practice might be disappointed more frequently than is necessary with the remark that 'it is just one of those things' or that 'no treatment is available'.

By which means should information be provided? The women themselves mention the general practitioner first of all. Since women of fertile age see their general practitioner fairly frequently (follow-up on oral contraception, pregnancy, etc.), his surgery is indeed a suitable place for providing information in pamphlets, brochures, etc. Provided the doctor himself is well-informed, he is the most suitable person to give the information. It is more effective to give a woman a brochure while adding an expert explanation than to 'display' pamphlets and brochures in the waiting-room.

Since women still have to be persuaded that vaginal discharge is not 'fate' but merely an inconvenience (often) amenable to treatment, much information will also have to be provided to a general public via more general channels. Information supplied via women's magazines is often mentioned by the women themselves. In this respect it is very important that the 'tone' of the imformation corresponds with the target group of the magazine in question: there are women's magazines for diverse age groups, for diverse degrees of

emancipation, for diverse social backgrounds and cultures, etc.
It is advisable to prepare an ample amount of basic material for the editors of these magazines, and to motivate them to write an informative article *themselves*. This is the best guarantee that the informative article will be consistent with the 'journalistic face' of the magazine involved.

References

1. Catterall RD. Diagnosis of vaginal discharge. Brit J Vener Dis 1970; 46: 122-124.
2. Gardner HL. The common vaginitides. J Ark Med Soc 1964; 60: 291-295.
3. Kålund Jensen H, Hansen PÅ, Blom J. Incidence of *Candida albicans* in women using oral contraceptives. Acta Obstet Gynecol Scand 1970; 49: 293-296.
4. Feichter GE, Nohlen M, Tauber PF. Bakterieller Fluor bei intrauteriner Kontrazeption. Fortschr Med 1979; 97: 1938-1940.
5. Barrett KF, Bledsoe S, Greer BE, Droegemueller W. Tampon-induced vaginal or cervical ulceration. Am J Obstet Gynecol 1977; 127: 332-333.
6. Lamberts H. Morbidity in general practice. Diagnosis related information from the Monitoring Project. Utrecht: Uitgevers-maatschappij Huisartsenpers B.V., 1984.
7. Continue Morbiditeits Registratie NUHI, Werkgroep Epidemiologie in de huisartspraktijk. Gewone ziekten. Nijmegen: Nijmeegs Universitair Huisartsen Instituut, 1980.
8. Nie NH, Hull CH, Jenkins JE, Steinbrenner K, Bent DH. Statistical package for the social sciences (2nd ed.). New York: McGraw-Hill, 1975.
9. Sturmans F. Epidemiologie en medische statistiek. Nijmegen: Dekker en Van de Vegt, 1975.
10. Stanley VC, Jones JB, Hurley R, Foulkes JEB, De Louvois J. Morbidity of the lower genital tract during pregnancy. J Clin Path 1975; 28: 736-740.
11. Ross SM, Hoosen AA, Sheik AI. Diagnosis and treatment of vaginal discharge in pregnancy. SA Med J 1980; 58: 757-759.
12. Van der Does CD. Beleving van gynaecologische klachten. Thesis. Oegstgeest: De Kempenaer, 1985.

VI DESCRIPTIVE LIGHT AND ELECTRON MICROSCOPY OF NORMAL AND CLUE CELL- POSITIVE DISCHARGE*

Willem I. van der Meijden[1], Henk Koerten[2], Wil van Mourik[3], and Willem C. de Bruijn[2,3]

Abstract

In women with clue cell-positive discharge (CCPD), light microscopical examination of the wet mount suggests a preference of bacteria for certain vaginal epithelial cells (VECs). To investigate this further, a light- and electron-microscopical study of patients and healthy controls was performed, with special emphasis on vitality and glycogen content of VECs and bacterial-epithelial cell interaction. Our study did not reveal morphologic differences between VECs of patients and controls. There was, however, a significant decrease in the percentages of vital and glycogen-containing VECs in CCPD ($p<0.001$), probably caused by an overgrowth of (anaerobic) bacteria. In CCPD vaginal bacteria preferably colonize vital VECs. This could account for the relatively low percentage of clue cells in this condition.

Introduction

Clue cell-positive discharge (bacterial vaginosis, *Gardnerella vaginalis*-associated vaginitis) is a common condition among women of childbearing age. It is characterized by whitish, homogeneous and malodorous discharge, without signs of vaginal inflammation [1]. The diagnosis 'clue cell-positive discharge' (CCPD) is based on the detection of clue cells in the wet mount preparation.

* Gynecol Obstet Invest (in press)

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands
[2] Laboratory for Electron Microscopy, University of Leiden, The Netherlands
[3] Department of Clinical Pathology, Laboratory for Experimental Electron Microscopy, Erasmus University, Rotterdam, The Netherlands

The wet mount from women with CCPD invariably shows that a minority of vaginal epithelial cells (VECs) are covered with bacteria. This finding has also been reported by other authors[2]. The aim of our study was to investigate this 'selective' bacterial adherence, thereby focusing on the vitality and glycogen content of VECs as well as on the morphology of bacterium-VEC interaction.

Materials and methods

Patients. Patients were recruited from women visiting the out-patient department of venereology to be checked for sexually transmitted diseases. All women taking part in this study were of childbearing age. Contraceptive method and phase of menstrual cycle were recorded.
Vaginal secretions. The vaginal fluid was collected from the lower valve of the speculum after its removal from the vagina. Part of it was studied immediately in the wet mount (suspension of vaginal discharge and normal saline). Vaginal discharge showing clue cells, VECs massively loaded with bacteria and obscured cell borders, was diagnosed as CCPD, while discharge showing no clue cells and a bacillary flora was considered normal (figures 1 and 2). A smear was also made and air-dried. The remaining fluid was suspended in 0.1 M phosphate buffered saline (PBS) of pH 7.4 and stored at 4-6°C until further processing, which generally took place within 3-6 hours.
Relative frequency of clue cells. The percentage of clue cells in the cell population of CCPD (40 patients) was determined by examining the wet mount preparation.
Vitality of VECs. Vitality was studied in 40 women with CCPD and in 40 controls (normal discharge) by means of the trypan blue exclusion method. For this purpose the vaginal discharge was mixed with 1% trypan blue in distilled water and immediately examined. VECs retaining trypan blue (blue nuclei) were classified as non-vital. The presence of clue cells was also noted.
Presence of glycogen. This was studied in the same 40 patients and 40 controls by staining the abovementioned air-dried smears with periodic acid Schiff reagent (PAS reaction). PAS positivity indicated the presence of glycogen. Since clue cells are difficult to recognize in (PAS)stained vaginal secretions we did not attempt to relate the PAS reaction to the VEC being a clue cell or not.
All three light microscopical procedures were done by examining 3x100 free-lying VECs per woman. The microscopical field was screened in an S-way(magnification 200x).

Fig. 1. Light microscopy of normal discharge (bar=100 μm). VECs (✸). Döderlein's bacilli (↟)

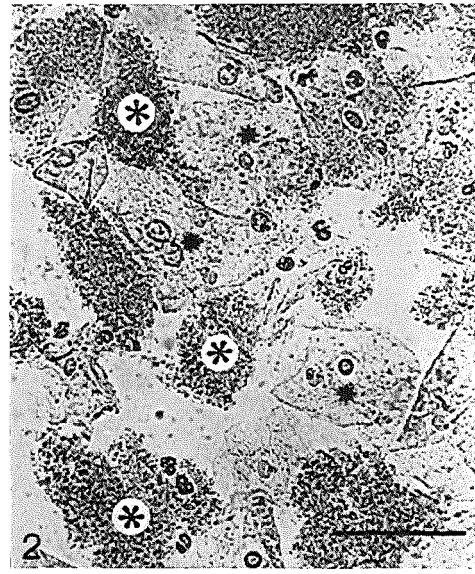


Fig. 2. Light microscopy of CCPD (bar=100 μm). Normal VECs (✸). Clue cells (✳)

Transmission electron microscopy (TEM). After suspension in PBS the vaginal fluid was 'washed' twice (by centrifugation at 2000 rpm for 5 minutes, followed by resuspension in PBS). The vaginal fluid was fixed in 1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 60 minutes and postfixed in 1% osmium tetroxide containing 0.05 M potassium ferrocyanide ($K_4Fe(CN)_6.3H_2O$). The latter fixative was chosen especially to visualize the glycogen in the VECs[3]. Specimens were dehydrated in acetone and embedded in Epon. Semi- and ultrathin sections were cut on an ultramicrotome; the latter were mounted on copper grids and stained with 2% aqueous uranyl acetate and lead citrate[4] prior to observation in a Philips 300 electron microscope (80 kV). Some of the aldehyde-fixed vaginal fluid was incubated with about 0.5% cationized ferritin (CFe-Miles) in cacodylate buffer (pH 7.3), followed by postfixation and further processing as described above.
Scanning electron microscopy (SEM). After suspension in PBS the vaginal fluid was 'washed' twice and fixed as described above.
After fixation, specimens were rinsed in the cacodylate buffer, dehydrated in a graded alcohol series, and critical point dried with liquid carbon dioxide. Samples were then mounted on stubs and coated with gold prior to observation in a Cambridge S 180 scanning electron microscope (at 15 kV in the secondary electron mode).
Treatment. Women with CCPD were treated with metronidazole, 2x500 mg daily, for one week [5]. Male partners were not routinely treated.
Statistics. For statistical testing of differences between two samples the t-test for independent samples was used. The relationship between the phenomenon clue cell and vitality of VECs was estimated and tested by means of the odds ratio [6].

Results

Relative frequency of clue cells. The mean percentage of clue cells in the VEC population of CCPD was 10.7 (± 3.4). Percentages were not influenced by the use of oral contraceptives or by the phase of the menstrual cycle.
Vitality of VECs. The mean percentage of vital VECs in normal discharge was 34.9 (± 18.0). In CCPD it was 3.7 (± 3.2). This difference is statistically significant ($p<0.001$). In case of CCPD vitality rates were not influenced by the use of oral contraceptives or by the phase of the menstrual cycle. In case of normal discharge, however, the mean percentage of vital VECs was significantly lower among women using oral contraceptives (29.4% vs.

Table I. Relation# between phenomenon clue cell and vitality of VECs

vitality of VECs +/–	number(%)	clue cell	no clue cell
+	441(3.7)	223(50.6)*	218(49.4)*
–	11,559(96.3)	769(6.7)**	10,790(93.3)**
VECs examined	12,000 (100)		

tested by odds ratio ($p<0.001$)
* percentage of vital VECs
** percentage of non-vital VECs

42.4%, $p<0.05$). The probability of a VEC being a clue cell was much lower (approximately 14x) in the case of non-vitality than in the case of vitality (table I).

Presence of glycogen. The mean percentage of PAS-positive VECs in normal discharge was 72.8 (±11.0). In CCPD it was 19.1 (± 13.5). This difference is statistically significant ($p<0.001$). In the specimens from 10 women with CCPD the percentage of PAS-positive VECs could only be roughly estimated due to the 'dirty' appearance of the microscopical field (numerous bacteria present). In case of CCPD PAS-positivity was not influenced by the use of oral contraceptives or by the phase of the menstrual cycle. In cases of normal discharge, however, the mean percentage of PAS-positive VECs was significantly higher among women using oral contraceptives. (77.8% vs. 68.8%, $p<0.01$). Ten women with CCPD were studied longitudinally, that is before treatment and after cure of the condition (table II).

Transmission electron microscopy. This technique enabled us to study cell organelles and glycogen granules in conjunction with the bacteria (figures 3 and 4). In order to establish the amount of glycogen in the VECs semiquantitatively, we examined 100 free-lying VEC cross-sections in the vaginal secretions of one woman with normal discharge and one woman with CCPD (figure 4a). Table 3 shows that in normal discharge some 70% of VEC cross-sections showed at least a moderate amount of glycogen, whereas in the case of CCPD this percentage was only 36.

Bacterial adherence was studied in relation to the cytochemical characterization of the cell coat by CFe. The visualization of surface coats of both bacteria and VECs is greatly enhanced by this marker, as demonstrated by figures 5-7. Bacteria in both normal discharge and CCPD seem to possess

Table II. Vitality and PAS positivity of VECs in CCPD, before treatment and after cure of the condition

		before treatment			after cure		
patient number	o.c. +/–	day of cycle	vital VECs (%)	PAS+ VECs (%)	day of cycle	vital VECs (%)	PAS+ VECs (%)
1	+	n.a.	0	<5	n.a.	38	73
2	+	n.a.	1	3	n.a.	11	86
3	+	n.a.	4	16	n.a.	53	86
4	+	n.a.	1	8	n.a.	24	61
5	+	n.a.	5	32	n.a.	42	80
6	+	n.a.	5	21	n.a.	16	84
7	–	11	6	34	25	27	62
8	–	21	2	4	12	14	68
9	–	23	6	49	5	27	74
10	–	9	1	<5	15	62	61
mean value			3.1	17.7		31.4	73.5

(%) mean percentage (3x 100 VECs examined)
o.c. oral contraceptives
n.a. not applicable

Table III. The amount of glycogen, semiquantitatively measured* in normal discharge and CCPD (VEC cross-sections)

Amount of glycogen	normal discharge	CCPD
no glycogen	10	43(8)
some glycogen	21	21(7)
moderate amount of glycogen	41	21(6)
much glycogen	28	15(1)
number of cross-sections examined	100	100(22)

* TEM, see also figure 4a
( ) number in parentheses = 'clue cells'

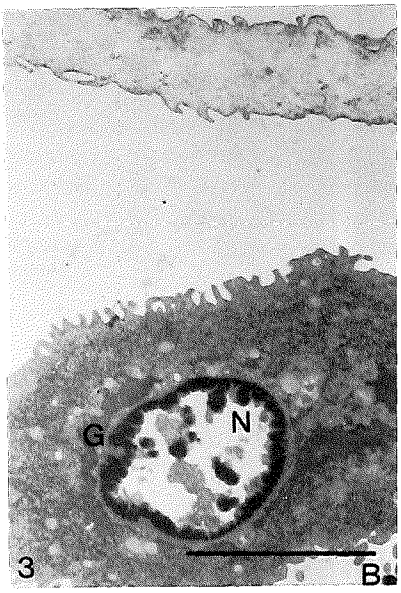
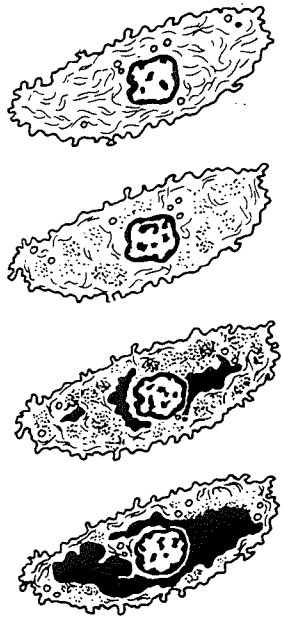


Fig. 3. and 4. TEM micrographs of normal discharge (bar=5 μm) and CCPD (bar=5 μm) respectively. Nucleus VEC (**N**). Glycogen (**G**). Tonofilaments (**T**). Attached bacteria (**B**)

Fig. 4a Semi-quantitative measurement of the amount of glycogen in VECs(TEM)



tonofilaments,
no glycogen

tonofilaments,
some glycogen

moderate amount
of glycogen

VEC loaded with glycogen,
tonofilaments in periphery

Fig. 5-7. TEM micrographs of normal discharge and CCPD. Glycogen (**G**). Tonofilaments (**T**). Attached bacteria (**B**)

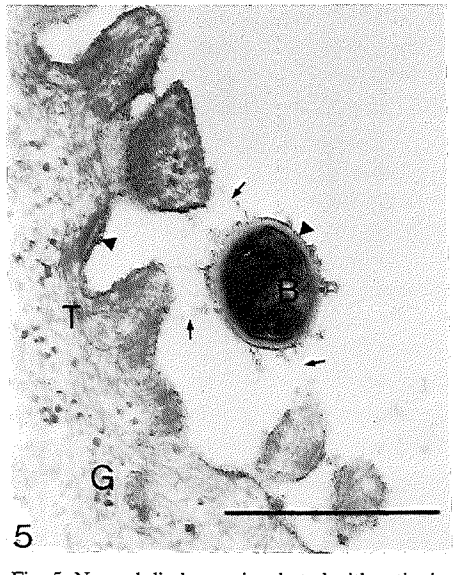


Fig. 5. Normal discharge, incubated with cationized ferritin (bar= 1μm). Ferritin particles on bacterial and epithelial cell surface (▲). Fiber-like structures, connecting bacterium with cell surface (↟)

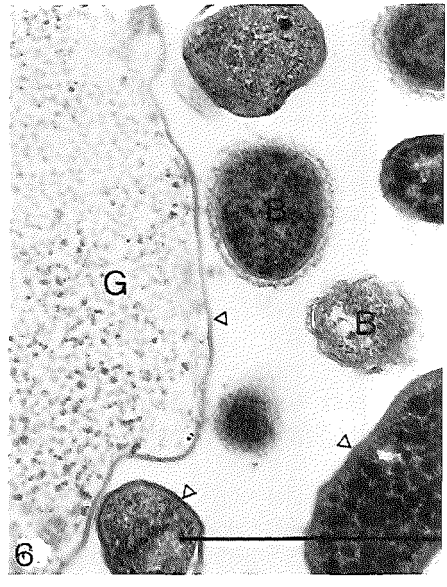


Fig. 6. CCPD, not incubated with cationized ferritin (bar= 1 μm). Absence of ferritin particles on cell surfaces (△)

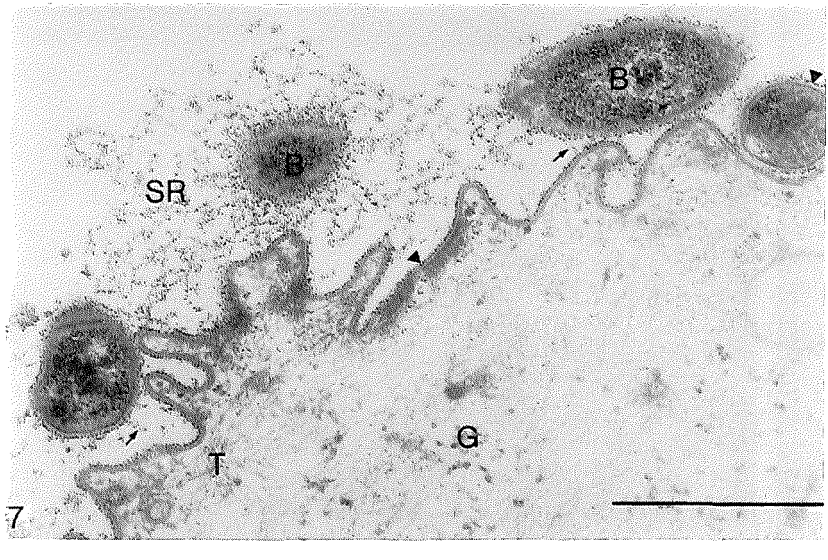


Fig. 7. CCPD, incubated with cationized ferritin (bar=1 μm). Ferritin particles on bacterial and epithelial cell surface (▲). Spider-like reticulum (**SR**)

Figs.8-11. SEM micrographs of normal discharge and CCPD

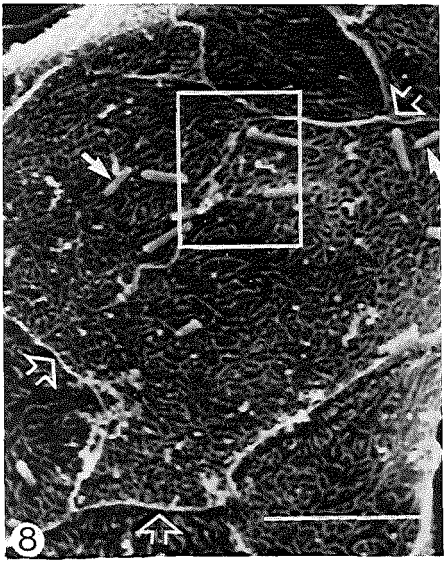


Fig. 8. Normal discharge (bar = 10 μm). Döderlein's bacilli (solid white arrouws). Rims on VEC, indicating attachment zones of formerly neighbouring VEGs (open white arrows)

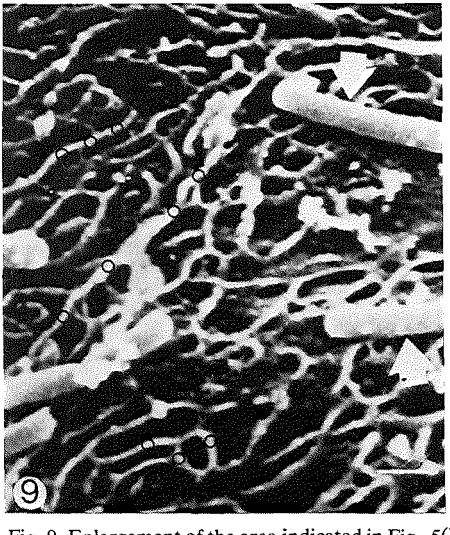


Fig. 9. Enlargement of the area indicated in Fig. 5(bar = 1 μm). Döderlein's bacilli (solid white arrows). Microridges (○)

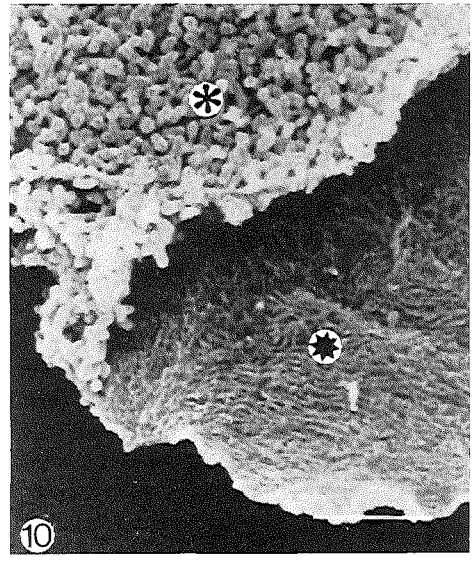


Fig. 10. CCPD (bar=5 μm). Clue cell (✳), partly covering normal VEC (✸)

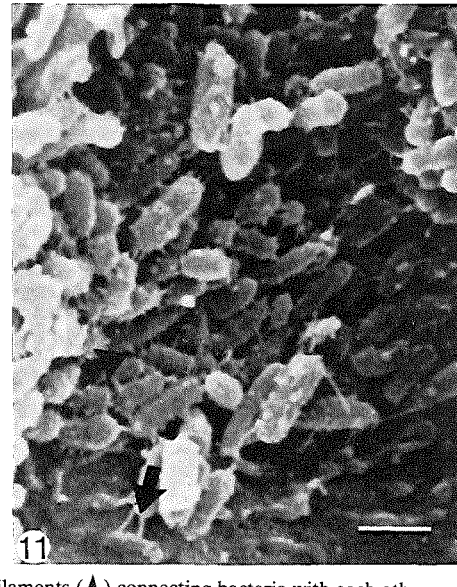


Fig. 11. CCPD (bar= 1 μm). Web-like filaments (▲) connecting bacteria with each other and with the cell surface

fiber-like structures with which they anchor to the epithelial surface (figures 5 and 7). The adherence of bacteria to each other is possibly also mediated through this 'hairy' component of their surface (figure 7). Apart from the number of attached bacteria and some type-dependant differences between bacteria (especially in length and cell wall), there were no major morphologic or cytochemical differences between bacterial adherence to VECs in normal vaginal discharge and CCPD.

Scanning electron microscopy. Figures 8-11 show SEM micrographs of normal discharge (figures 8 and 9) and CCPD (figures 10 and 11). In normal discharge relatively few bacteria, presumably lactobacilli, are attached to the VECs. The surface structure of the VECs can be clearly seen. In the case of CCPD some VECs are massively loaded with bacteria. These bacteria seem to be present in a layer on the cell surface (figure 10). Bacteria attached to the VECs sometimes seem to be connected with each other by very thin, web-like filaments (figure 11). The surface structure of clue cells cannot be seen, or only partially. On the other hand, VECs adjacent to clue cells apparently have a normal surface structure (figure 10).

Discussion

In the literature, data concerning the characteristics of VECs in CCPD are scarce. Fleury [7] initially thought that *G. vaginalis* preferred cells with higher glycogen content, but later found that this was not the case. He also stated that 'the reason why *G. vaginalis* attaches to some cells and not to others is presently unknown'.

Our light-microscopical study indicates that the vast majority of VECs in CCPD look normal if not tested for vitality and glycogen content, showing well-defined borders and clearly visible nuclei.

The percentage of clue cells, representing bacterial adherence in optima forma, was not influenced by the use of oral contraceptives or by the phase of the menstrual cycle. *In vitro* experiments yield conflicting data concerning the role of hormonal influences on bacterial adherence to VECs[8,9].

A possible explanation of the decrease in vitality of VECs in the case of CCPD is that certain fermentation products of anaerobic bacteria, e.g. amines[10], damage the integrity of the cell wall either as 'short'-range (bacteria on cell surface) or as 'long'-range effect (bacteria in intercellar fluid). As shown in table II, 'CCPD-bacteria' preferably colonize vital VECs. However, non-vital VECs could also be identified as clue cell, albeit much less frequently. *In vitro* experiments have shown that vitality of VECs is not

necessary for bacterial adherence to these cells[11]. The lower vitality rate of VECs in normal discharge of women using oral contraceptives is possibly caused by the fact that they induce a 'pseudo-pregnancy' state of the vaginal epithelium [12]. The higher glycogen content of the vaginal mucosa under these circumstances indirectly favors the growth of lactobacilli, which are in turn responsible for the so-called 'bacterial cytolysis' [13].

The mean percentage of VECs containing glycogen (PAS-positive) was significantly lower in women with CCPD, than in women with normal discharge. The huge number of bacteria (especially anaerobes) in case of CCPD is probably responsible, consuming a 'large' amount of sugars and, indirectly, glycogen. In both normal discharge and CCPD we did not find a significant difference in PAS-positivity between pre- and postovulatory specimens. The glycogen content of the vaginal fluid seems to be rather constant under different conditions [14]. The higher percentage of PAS-positive VECs in normal discharge of women using oral contraceptives probably reflects the pseudo-pregnancy state of the vaginal epithelium, as mentioned earlier.

The presumption that the affinity of bacteria to VECs has something to do with the glycogen content of VECs seems plausible. It is generally agreed that bacteria harboring the vagina need simple sugars such as glucose which is derived from glycogen, for their metabolism. Adherence of bacteria to the VEC surface possibly creates a suitable environment in which bacterial enzymatic action on 'food particles' can take place in a more efficient way, than in the more or less 'diluted' intercellular fluid. However, in normal discharge for example one can see numerous free-floating bacilli between the VECs, with relatively few bacilli attached to VECs. Direct contact between bacteria and VECs therefore does not seem a prerequisite for bacterial metabolism.

From table III one may perhaps conclude that the presence of glycogen is not necessary for (massive) bacterial adherence and clue cell formation. One could, however, also suggest that the data indicate the 'consumption' of glycogen by adhering bacteria. The authors hypothesize that the VECs in CCPD go through several stages: 1. preadherence, vital, much glycogen, 2. adherence, vital, less glycogen, 3. adherence, non-vital, no glycogen and 4. postadherence, non-vital, no glycogen. Bacterial 'disconnection' seems a plausible explanation of the fact that a relatively low percentage of VECs are found in stage 3. The fact that twice as many VEC cross-sections in CCPD as in normal discharge showed bacterial adherence, demonstrates that, on the whole, bacterial adherence is much more common in CCPD.

Most authors studying bacterial glycocalyces have used ruthenium red, a

specific stain for polyanionic structures [15]. We visualized bacterial and epithelial cell coats with CFe (figures 5 and 7). In our opinion CFe is to be preferred , giving a better contrast. Furthermore, it seems difficult to produce ruthenium red of 'standard quality'.

Glycocalyx material consists of exopolysaccharides that contain approximately 99% water[16]. Following dehydration for ultrastructural research, the glycocalyx is reduced to a sharply condensed residue on the cell surface or a spider-like reticulum between cells[17]. It could therefore well be that the fiber-like structures we visualized in figures 5 and 7 merely represent the partially condensed glycocalyces. Demonstration of the real dimensions of the glycocalyx is believed to require stabilization of the polysaccharide coat with lectins or specific antibodies[18,19].

Our SEM data suggest that there are no morphologic differences between VECs in normal vaginal discharge and CCPD. In both situations microvilli and microridges can be seen (figures 8-11), structures which seem to be important for the integrity of the epithelium [20]. In the case of clue cells no surface structures are visible, the surface being covered with bacteria (figure 10).

It was sometimes possible clearly to distinguish web-like filaments connecting bacteria with each other (figure 11). These filaments are probably artefacts representing the dehydrated, condensed bacterial glycocalyces. They should be carefully distinguished from pili, structures that enhance the adhering capacity of many Gram-negative bacteria, which are 7-10 nm in diameter and exceptionally difficult to resolve by scanning electron microscopy [21].

From our study, which is the first to present *in vivo* data about bacterial adherence to VECs, it can be concluded that there is indeed a selective bacterial adherence to VECs in cases of CCPD. *Gardnerella vaginalis* and other 'CCPD-bacteria' preferably colonize vital VECs, apparently because this creates a favorable micro-environment. The significant decrease in the percentage of both vital and glycogen containing VECs in CCPD is probably caused by massive overgrowth of especially anaerobic bacteria. The great difference in adhering capacity between lactobacilli and 'CCPD-bacteria' could be glycocalyx-dependant. We advocate the use of the 'clue cell model' in future research work.

References

1. Van der Meijden, W.I. : Clinical aspects of *Gardnerella vaginalis*-associated vaginitis. A review of the literature. Scand. J. Urol. Nephrol. suppl. 86: 135-141 (1985).
2. Vontver, L.A. ; Eschenbach, D.A.: The role of *Gardnerella vaginalis* in nonspecific vaginitis. Clin. Obstet. Gynecol. 24: 439-460 (1981).
3. De Bruijn, W.C.; Den Breejen, P.: Glycogen, its chemistry and morphological appearance in the electron microscope. II. The complex formed in the selective contrast staining of glycogen. Histochem. J. 7: 205-229 (1975).
4. Huxley, H.E.; Zubay, G.: Preferential staining of nucleic acid-containing structures for electron microscopy. J. biophys. biochem. Cytol. 11: 273-296 (1961).
5. Pheifer, T.A. ; Forsyth, P.S.; Durfee, M.A.; Pollock, H.M.; Holmes, K.K.: Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. New Engl. J. Med. 298: 1429-1434 (1978).
6. Fleiss, J.L. : Methods for rates and proportions, 2nd ed. John Wiley & Sons, New York, 1981.
7. Fleury, F.J.: The clinical signs and symptoms of *Gardnerella*-associated vaginosis. Scand. J. Infect. Dis. suppl. 40: 71-72 (1983).
8. Forslin, L.; Danielsson, D.: In vitro studies of the adherence of *Neisseria gonorrhoeae* and other urogenital bacteria to vaginal and uro-epithelial cells, with special regard to the menstrual cycle. Gynecol. Obstet. Invest. 11: 327-340 (1980).
9. Sobel, J.D.; Schneider, J.; Kaye, D.; Levison, M.E.: Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect. Immun. 32: 194-197 (1981).
10. Chen, K.C.S.; Forsyth, P.S.; Buchanan, T.M.; Holmes, K.K.: Amine content of vaginal fluid from treated and untreated patients with nonspecific vaginitis. J. Clin. Invest. 63: 828-835 (1979).
11. Zawaneh, S.M. ; Ayoub, E.M.; Baer, M.; Cruz, A.C.; Spellacy, W.N.: Factors influencing adherence of group B streptococci to human vaginal epithelial cells. Infect. Immun. 26: 441-447 (1979).
12. Catterall, R.D.: *Candida albicans* and the contraceptive pill. Lancet *ii*: 830-831 (1966).
13. Wied, G.L.; Christiansen, W.: Die Zytolyse von Epithelien des Vaginalsekretes. Geburtsh. u. Frauenheilkd. 13: 986-995 (1953).
14. Stancey, T.A.; Timothy, M.M.: Studies of introital colonization in women with recurrent urinary infections. III. Vaginal glycogen concentrations. J. Urol. 114: 268-270 (1975).
15. Luft, J.H.: Ruthenium red and ruthenium violet. I. Chemistry, purification, methods for use for electron microscopy, and mechanism of action. Anat. Rec. 171: 347-368 (1971).
16. Sutherland, I.W.: Bacterial exopolysaccharides: their nature and production. In: Surface carbohydrates of the prokaryotic cell. I.W. Sutherland (ed.) Academic Press, New York, p. 27-96 (1977).
17. Lambe, D.W.; Mayberry-Carson, K.J.; Ferguson, K.P.; Costerton, J.W.: Morphological stabilization of the glycocalyces of 23 strains of five *Bacteroides* species using specific antisera. Can. J. Microbiol. 30: 451-460 (1984).
18. Birdsell, D.C.; Doyle, R.J.; Morgenstern, M.: Organization of teichoic acid in the cell wall of *Bacillus subtilis*. J. Bacteriol. 121: 726-734 (1975).
19. Bayer, M.E.; Thurow, H.: Polysaccharide capsule of *Escherichia coli*: microscope study of its size, structure and sites of synthesis. J. Bacteriol. 130: 911-936 (1977).
20. Llanes, A.T.; Farre, C.B.; Ferenczy, A.; Richart, R.M.: Scanning electron microscopy of normal exfoliated squamous cervical cells. Acta Cytol. 17: 507-509 (1973).

21. Costerton, J.W.; Irvin, R.I.; Cheng, K.-J.: The role of bacterial surface structures in pathogenesis. Crit. Rev. Microbiol. 8: 303-338 (1981).

VII TREATMENT OF NON-SPECIFIC VAGINITIS WITH A SINGLE DOSE OF TINIDAZOLE*

Willem I. van der Meijden

Abstract

In a double-blind randomized trial, twenty-six patients with non-specific vaginitis were treated with tinidazole/placebo (4x500 mg in a single dose). Thirteen patients received placebo, of whom one was cured. Thirteen patients received tinidazole, of whom six were cured. The nineteen non-responders received tinidazole (4x500 mg in a single dose), which resulted in the cure of eight patients (seven from the original placebo group and one from the original tinidazole group). Of the eleven patients not responding to tinidazole, seven were successfully treated with metronidazole (250 mg 4 times daily for one week).

Introduction

In 1955 Gardner and Dukes [1] introduced the term *'Haemophilus vaginalis vaginitis'*. In the vast majority of cases so-called 'non-specific' vaginitis was supposed to be caused by *Haemophilus vaginalis*, a Gram-negative coccobacillus.

In the past 25 years numerous articles on this subject have been published. The taxonomic position and the pathogenicity of the micro-organism have been topics ever since. In 1980 Greenwood and Pickett [2] proposed to call the bacterium *Gardnerella vaginalis*. This name seems to have found general acceptance. There are still divergent views on the pathogenicity of *Gardnerella vaginalis*. Some investigators, e.g. McCormack[3] and Levison[4], consider *G.vaginalis* a normal inhabitant of the vagina. Most authors, however, think of *G.vaginalis* as a more or less pathogenic micro-organism.

In recent years anaerobic bacteria have also been connected with non-specific vaginitis (NSV). According to some investigators there might be a

* *Scand J Infect Dis 1983; suppl 40: 85-89*

symbiotic relationship between *G.vaginalis* and anaerobes[5,6]. Spiegel et al.[7] made a quantitative bacteriological study of vaginal secretion in NSV patients. *G.vaginalis* and anaerobes were isolated in a significantly higher concentration from vaginal secretion in NSV patients than from that in controls.
Non-specific vaginitis should preferably be treated by systemic administration of drugs. In the past, ampicillin and tetracyclines have often been used with varying but generally poor results. A real break-through in the treatment of NSV took place with the introduction of metronidazole for this indication. Pheifer et al.[5] treated eighty-one patients with metronidazole , 500 mg by mouth twice daily for seven days. Eighty patients showed clinical improvement and eradication of *G.vaginalis* from the vaginal secretion. Other authors claim good results with the same regimen.
This study was undertaken to compare single-dose therapy with tinidazole (a well-known trichomonacidal drug among the nitro-imidazole combinations) with one-week metronidazole therapy. All this from the point of view of patient compliance and cost effectiveness.

Material and methods

Twenty-six patients with NSV participated in this study. The diagnosis 'non-specific vaginitis' was made when the microscopic preparation of the vaginal secretion did not show pseudo-hyphae or *Trichomonas vaginalis* and when the secretion met at least 2 of the following criteria: homogeneous consistency, positive amine test, pH >4.5, clue cells present (according to Spiegel et al.[7]). The vaginal secretion was considered normal when only 1 or none of the NSV criteria was met.
The women in this study were all of childbearing age, the average age being 34 (21-49). They did not use oral contraceptives and had regular periods. In the month before visiting the out-patient department the patients did not use antibiotics. The women did not have sexual contact for at least 3 days before the gynaecological examination. At the first visit the gynaecological history was recorded. During the follow-up visits, taking place at 4-week intervals, questions were asked only about complaints related to vaginal discharge and about possible side effects of the drug prescribed. Questions about multiple sex partners were not asked routinely, but only in the case of treatment failure. When the patient was examined with a bi-valve speculum, the macroscopic aspect of cervix, vagina and vaginal secretion was noted. The pH was measured half-way down the right lateral vaginal wall and some secretion was collected for microscopic and bacteriological study.

When the vaginal secretion was examined under the microscope, the presence of clue cells, bacterial lumps, leucocytes and parabasal cells was noted. After removal of the bi-valve speculum, a small amount of KOH 10% was dripped onto the lower valve of the speculum, where some secretion is always found. When a rotten-fish odour arose, the amine test was recorded as positive.
The material for bacteriological study was transported in Stuart's medium and inoculated into the different media within 1 to 3 hours of collection. A semi-quantitative culture method was used in which bacterial growth is recorded as sporadic, +, ++ or +++.
For isolation of anaerobes and *G.vaginalis*, incubation took place in the GASPAK system. Identification of the anaerobes was done with the API-20A system. In culturing *G.vaginalis* a special medium was used: Columbia Agar CNA (Gibco Bio-cult Diagnostics Ltd., Scotland) with an upper layer of 5% human blood. Gram-negative bacilli, causing β-haemolysis in this medium, were interpreted as *G.vaginalis* when the oxidase and catalase tests were negative and growth was delayed by hydrogen peroxide.
Initially the patients and their consorts were treated with tinidazole/placebo (4x500 mg in a single dose). This treatment was double-blind/randomized. When a second course had to be prescribed, we gave tinidazole in all cases (4x500 mg in a single dose). In the third and following courses metronidazole was used (4x250 mg daily for one week).

Results

Of twenty-six patients with NSV, only five (20%) spontaneously went to their family doctor or gynaecologist, complaining of 'abnormal discharge'. Three patients did not have any complaints. The other eighteen women all had specific complaints, but only when the doctor asked for them. Most of the women had a profuse and malodorous vaginal discharge. About 80% of the patients was aware of malodour (Table I). After normalization of the vaginal secretion most of the patients were without complaints. Two patients still had a 'profuse discharge'; one patient had not noticed any improvement at all.
At the initial visit the vaginal secretion of seventeen (65%) of the NSV patients met all 4 criteria (homogeneous consistency, pH >4.5, positive amine test and clue cells present). The secretion of eight of them met 3 criteria. The secretion of only one of them met 2 criteria. After cure the

Table I. Complaints at initial visit

Profuse vaginal secretion		2
Profuse and malodorous vaginal secretion		16
Malodorous vaginal secretion		5
No complaints		3
Vulvar itching	1	additional complaints
Sore feeling	3	additional complaints

* The vaginal culture of one of these patients showed sporadic growth of *Candida albicans*.

vaginal secretion of most of the patients was white and curdy. It was never foul-smelling and the amine test was never positive.

The average vaginal pH before treatment was 5.15 (± 0.42). After cure the vaginal pH was significantly lower: 4.20 (± 0.38). Wilcoxon test $p<0.01$.

The patients and their (regular) consorts were treated with tinidazole/placebo (4x500 mg in a single dose). Of thirteen patients initially treated with tinidazole, six were cured (45%). The seven unsuccessfully treated patients received a second course of tinidazole. Only one was cured. Of thirteen patients initially treated with placebo, one was cured. The twelve non-responders were treated with tinidazole, and seven of them were cured (60%). Eleven patients not responding to tinidazole (one or two courses) were treated with metronidazole (4x250 mg daily for one week). Seven patients (65%) were cured after one course. Two patients needed two courses, one patient even needed three. One patient was not cured after two courses of tinidazole and one course of metronidazole. She, however, had no complaints and further treatment was not considered necessary. Two patients were reinfected within 1 to 2 months of being cured. Their male consorts admitted that they had had other sexual contacts. The therapy caused some side effects, which were not serious (Table II). Having side effects was no reason to stop therapy before the end of the course, except for one patient. She and her husband stopped taking metronidazole after four days, because of serious headache.

G. vaginalis and obligate anaerobes were isolated more frequently from the vaginal secretion of NSV patients before treatment than after cure. In contrast with the results of other studies[7,8] such a difference could not be proven for lactobacilli (Fig.1). Differences in culture- and/or identification method are probably mainly responsible for this. The results of the aerobic and anaerobic cultures (first visit) are listed in Tables III and IV. So far as the

Table II. Side effects therapy (first prescription)

	Patient		(Regular)male consort	
Placebo (n=13)	No complaints	13	No complaints	13
Tinidazole (n=25)	No complaints	21	No complaints	25
	Nausea	2		
	Metallic taste	1		
	Generalized pruritus	1		
Metronidazole (n=11)	No complaints	7	No complaints	8
	Nausea	2(1)*	Nausea	2
	Headache	2	Headache	1

* This patient had generalized pruritus after taking tinidazole

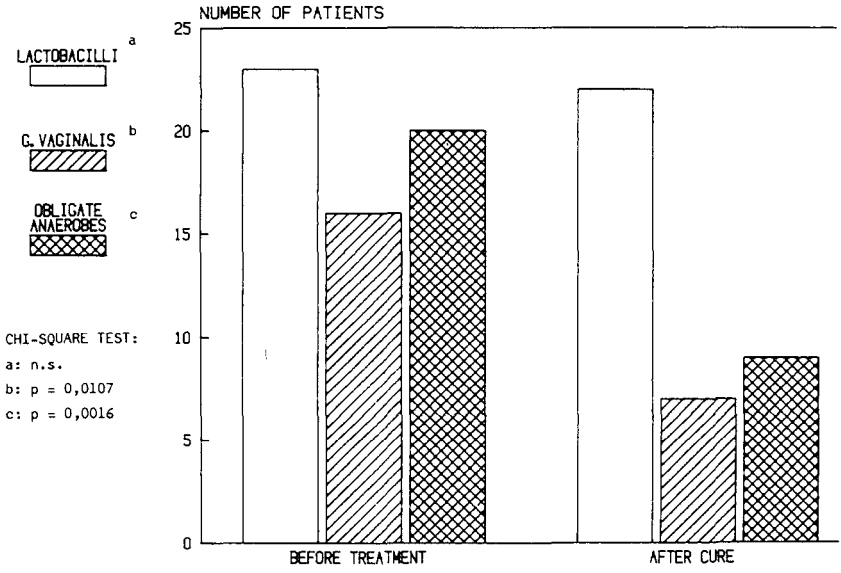


Fig.1. Isolation of lactobacilli, *G.vaginalis* and obligate anaerobes (one or more species) from patients with NSV, before treatment and after cure (n=25)

Table III. Facultative anaerobes isolated from vaginal secretion of twenty-six NSV patients (first visit)

	Bacterial growth (semi quantitative)				
	Spor.	+	++	+++	No. of isolates
Lactobacillus spp.	–	4	12	8	24
Gardnerella vaginalis	–	–	3	13	16
coagulase-negative staphylococci	8	1	1	–	10
β-haemolytic streptococci *Lancefield Group B*	2	–	–	–	3
α-haemolytic streptococci	–	1	1	1	3
Escherichia coli	–	2	–	–	2
Non-haemolytic streptococci	–	1	–	–	1
Streptococcus faecalis	–	1	–	–	1

Table IV. Obligate anaerobes isolated from vaginal secretion of twenty-six NSV patients (first visit)

	Bacterial growth (semiquantitative)				
	Spor.	+	++	+++	No. of isolates
Bacteroides spp.	–	4	8	4	16
Peptostreptococci	–	1	6	7	14
B.melaninogenicus	–	4	6	–	10

obligate anaerobes are concerned, *Bacteroides* species and *Peptostreptococci* were the predominant bacteria.

Discussion

Non-specific vaginitis (*G.vaginalis*-associated vaginitis) is generally looked upon as a mild condition, which on the other hand can be very troublesome to the patient and her sexual partner.

Perhaps fewer than half the women with the infection volunteer complaints of discharge and odour [9]. In our study only 5 patients (20%) spontaneously went to their family doctor or gynaecologist complaining of 'abnormal discharge'. Many women accept it as the normal status. The use of minipads

could also be responsible for patient delay. The taboo still connected with genitals and sexuality should not be underestimated either. Malodour is the commonest complaint (Table I). Other studies confirm this. Sometimes the partner is more aware of this disagreeable odour than the patient herself. Using the criteria according to Spiegel et al.[7], the diagnosis 'non-specific vaginitis' can be made easily. The microscope is the most valuable diagnostic aid, the clue cell being a highly characteristic feature.

In our study data were collected indicating a 50% effectiveness of a single dose of tinidazole (4x500 mg) in non-specific vaginitis. Seven of eleven patients not responding to tinidazole were successfully treated with metronidazole, 4x250 mg daily for one week. This cure rate (65%) is rather low as compared with that in other studies (over 90%). A possible explanation could be that it concerned selected patients, not responding to tinidazole (one or two courses). Bardi et al.[10] claimed a cure rate of 86% with a single dose of tinidazole (4x500 mg). Thirty-five patients with *G.vaginalis* infection were treated this way and thirty patients recorded disappearance of all symptoms within 48 hours. During the First Sexually Transmitted Diseases World Congress (San Juan, 1981) Balsdon advocated the use of a single 2-gramme dose of metronidazole. According to the results of our study one should not expect much higher cure rates than with tinidazole, the only way to find out being a double-blind randomized trial.

Pheifer et al.[5] showed one-week therapy with metronidazole to be very effective. Malouf et al.[11] used the same regimen. Metronidazole proved to be effective in twenty of twenty-one couples (90.9%) treated. Balsdon et al.[12] did a controlled trial of treatment with *G.vaginalis* vaginitis. All but one of seventeen patients treated with metronidazole (400 mg twice daily for one week) were cured.

There is generally a strong tendency to prescribe short and whenever possible single-dose therapies. This must be stimulated when there is enough evidence that cure rates are acceptable. Patient compliance and cost effectiveness could be influenced in a positive way.

In view of the discouraging results with single-dose tinidazole therapy, non-specific vaginitis should preferably be treated with the regimen introduced by Pheifer et al. [5]: metronidazole 500 mg twice daily for one week.

Gardnerella vaginalis and obligate anaerobes were isolated significantly more often from the vaginal secretion of NSV patients before treatment than after cure. They undoubtedly play a major role in the pathogenesis of this condition.

References

1. Gardner, H.L. & Dukes, C.D.: *Haemophilus vaginalis* vaginitis. Am J Obstet Gynecol 69: 962, 1955.
2. Greenwood, J.R. & Pickett, M.J.: Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus, Gardnerella: *Gardnerella vaginalis* (Gardner and Dukes) comb. nov. Int J Syst Bacteriol 30: 170-178, 1980.
3. McCormack, W.M., Hayes, C.M., Rosner, B.C., Evrard, J.R., Crocket, V.A., Alpert, S., Zinner, S.H.: Vaginal colonization with *Corynebacterium vaginale (Haemophilus vaginalis)*. J Infect Dis 136: 740-745, 1977.
4. Levison, M.E., Trestman, I., Quach, R., Sladowski, C. & Floro, C.N.: Quantitative bacteriology of the vaginal flora in vaginitis. Am J Obstet Gynecol 133: 139-144, 1979.
5. Pheifer, T.A., Forsyth, P.S., Durfee, M.A., Pollock, H.M. & Holmes, K.K.: Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. NewEngl J Med 298: 1429-1434, 1978.
6. Chen, K.C.S., Forsyth, P.S., Buchanan, T.M. & Holmes, K.K.: Amine content of vaginal fluid from untreated and treated patients with non-specific vaginitis. J Clin Invest 63: 828-835, 1979.
7. Spiegel, C.A., Amsel, R., Eschenbach, D., Schoenknecht, F. & Holmes, K.K.: Anaerobic bacteria in non-specific vaginitis. New Engl J Med 303: 601-607, 1980.
8. Taylor, E., Blackwell, A.L., Barlow, D. & Phillips, I.: *Gardnerella vaginalis*, anaerobes, and vaginal discharge. Lancet *i*: 1376-1379, 1982.
9. Gardner, H.L.: *Haemophilus vaginalis* vaginitis after twenty-five years. Am J Obstet Gynecol 137: 383-391, 1980.
10. Bardi, M., Lasala, L., Manenti, G. & Mattioni, D.: Metronidazole for non-specific vaginitis. Lancet *i*: 1029, 1981.
11. Malouf, M., Fortier, M., Morin, G. & Dube, J.- L.: Treatment of *Haemophilus vaginalis* vaginitis. Obstet Gynecol 57: 711-714, 1981.
12. Balsdon, M.J., Taylor, G.E., Pead, L. & Maskell, R.: *Corynebacterium vaginale* and vaginitis: a controlled trial of treatment. Lancet *i*: 501-504, 1980.

VIII AMOXYCILLIN, AMOXYCILLIN-CLAVULANIC ACID AND METRONIDAZOLE IN THE TREATMENT OF CLUE CELL - POSITIVE DISCHARGE. A COMPARATIVE CLINICAL AND LABORATORY STUDY*

Willem I. van der Meijden[1], Peter Piot[2], Suzanne M. Loriaux[3] and Ernst Stolz[1]

Summary

In an open study 30 women with clue cell-positive discharge (CCPD) were treated with amoxycillin, amoxycillin-clavulanic acid or metronidazole (10 women in each group). Patients were frequently monitored, namely at day 2, 4, 9 and 15. Clinical and microscopical parameters were used to assess treatment efficacy. Gas-liquid chromatography of vaginal secretions was also performed. The succinate-lactate ratio correlated very well with the clinical findings. All patients treated with metronidazole were cured by day 4, suggesting that for example a three-day course is as effective as the standard seven-day regimen. Amoxycillin-clavulanic acid was moderately effective in the long term, resulting in the cure of seven patients at day 15. Amoxycillin had to be considered less effective, establishing normal secretions in only four patients. Gastro-intestinal complaints, although common in patients treated with amoxycillin-clavulanic acid, were generally mild and no reason to discontinue treatment. In conclusion, we consider amoxycillin-clavulanic acid a reasonable alternative in the treatment of CCPD. Its use should, however, be reserved for pregnant women and those showing metronidazole intolerance.

* J Antimicrob Chemother (in press, modified version)

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands
[2] Department of Medical Microbiology, Institute of Tropical Medicine, Antwerp, Belgium
[3] Medical Department, Beecham Research Laboratories, Amstelveen, The Netherlands

Introduction

The treatment of bacterial vaginosis, a vaginal disease entity also described as clue cell-positive discharge (Van der Meijden, 1985), has long been controversial, as recently shown in a review dealing with this subject (Piot, 1985). One might consider the introduction of metronidazole for this condition a break-through (Pheifer et al., 1978). This nitro-imidazole derivative has been shown to be active against clue cell-positive discharge (CCPD) in various regimens (Høvik, 1983; Jerve, Qvigstad & Eng, 1983).
Since CCPD is perhaps the most prevalent vaginal infection, and generally a clinically mild disease without complications, treatment should be as innocuous as possible.
Although there is no convincing evidence that metronidazole has teratogenic properties in human beings (Roe, 1983), the drug should be used only reluctantly during pregnancy. The development of an effective treatment which can be safely used during pregnancy seems therefore important.
Our pilot study was undertaken in order to compare the efficacy of amoxycillin, a broad-spectrum penicillin, and amoxycillin plus clavulanic acid, a combination which is active against aerobic and anaerobic ß-lactamase-producing bacteria, with that of metronidazole in the treatment of CCPD.

Materials and methods

Study population. Patients were selected from women visiting the outpatient department of venereology in order to be checked for sexually transmitted diseases. They were enrolled if they had malodorous discharge with a positive amine test and if the wet mount showed clue cells in the absence of *Trichomonas vaginalis* or pseudo-hyphae. Patients were excluded if they had used antibiotics within the preceding two weeks, had multiple sexual partners, or did not use oral contraceptives. Women with non-odorous, floccular secretions, showing Döderlein's bacilli in the wet mount and otherwise fulfilling the inclusion criteria, served as controls.
They were fully assessed, clinically and bacteriologically, at day 1 (intake). Enrollment was continued until there were 10 evaluable patients in each of the three treatment groups and the control group.
Treatment. Patients were allocated to one of three therapeutic regimens: amoxycillin 500 mg capsules t.i.d. for one week, amoxycillin-clavulanic acid 625 mg tablets t.i.d. for one week or metronidazole 500 mg tablets b.i.d. for one week. Treatment had to commence on the day of the first consultation. Sexual partners were not routinely treated. We did not advise patients to

abstain from sexual contact during the study period.
Follow-up. Patients were frequently monitored, namely at day 2 (approximately 24 hours after the intake), 4, 9 and 15. At each visit several anamnestic and clinical parameters were recorded. Adverse effects were recorded only if volunteered by the patient.
Microbiological methods. At each session a small sample of vaginal discharge was suspended in normal saline and deep-frozen (-20° C) for gas-chromatographic analysis on non-volatile organic acids (succinate, lactate) in due time. Gas-liquid chromatography was performed as described previously (Piot et al., 1982).
Cultures for *Neisseria gonorrhoeae* (cervix, urethra), *Chlamydia trachomatis* (cervix), *T.vaginalis* (posterior fornix) and *Candida* species (lateral vaginal wall) were taken at day 1 (intake), 9 and 15. *N. gonorrhoeae* strains were isolated and identified on a selective gonococcal agar medium using standard procedures. *C.trachomatis* was cultured on a Hela 229 monolayer (pretreated with DEAE-dextran in Hank's balanced salt solution (30 mcg/ml)) grown in a 96 well microtitre tissue culture plate (Falcon no. 3070). After inoculation the plates were incubated for 48 hours at 37° C in a CO_2 incubator. Detection of chlamydial inclusions was carried out with the aid of fluorescein monoclonal antibodies (SYVA inc.). *T. vaginalis* was cultured in Ringer-Cysteine-Liver-Glucose medium for three days under aerobic conditions at 37°. *Candida* species were cultured on Sabouraud's agar for seven days under aerobic conditions at 25° C.
In monitoring treatment efficacy, we distinguished between 'improvement' and 'cure', the definitions of which are shown below.

Improvement	*Cure*
non-odorous discharge	non-odorous discharge
negative amine test	negative amine test
clue cells absent	clue cells absent
Döderlein's bacilli *absent*	Döderlein's bacilli *present*

In women whose vaginal secretions did not meet all the criteria of 'improvement' or 'cure', treatment efficacy was indicated as 'indefinite'. The conclusion 'not cured' indicates persistence or recurrence of the 'fullblown' picture of CCPD, i.e. malodorous, homogeneous discharge, positive amine test and clue cells but no Döderlein's bacilli in the wet mount.

Results

A total of 65 women were enrolled. We only report data of the women who were fully evaluable (4x10). The mean age of patients and controls did not

Table I. Vaginal and vulvar complaints in women with CCPD and controls, at day 1 (intake)

	patients (n=30)	controls (n=10)	p-value
Complaints			
profuse discharge	12(40)	2(20)	0.44*
odorous discharge	11(37)	0(0)	0.04*
pruritus and/or irritation	3(10)	1(10)	1
No complaints	14(47)	8(80)	0.08*

( ) number in parentheses = percentage
* Fisher's exact test

Table II. Some clinical and laboratory findings in women with CCPD and controls, at day 1 (intake)

	patients (n=30)	controls (n=10)	p-value
Amount of discharge			
scant	14(47)	5(50)	
moderate	14(47)	5(50)	
profuse	2(7)	0(0)	0.70*
Vaginal pH			
≤4.4	2(7)	10(100)	
>4.4	28(93)	0(0)	<0.001**
Leucocytes wet mount			
none	2(7)	0(0)	
few	10(33)	7(70)	
moderate	8(27)	3(30)	
many	10(33)	0(0)	0.09*
S/L ratio			
<0.40	2(7)	10(100)[a]	
≥0.40	28(93)[b]	0(0)	<0.001**

( ) number in parentheses = percentage
* X^2 test
** Fisher's exact test
[a] no succinate detectable in 1 case
[b] no lactate detectable in 12 cases
n.d. = not done

differ significantly (27.5 yrs. versus 24.6 yrs.). As shown in table I, only 53% of the women with CCPD reported complaints, among which profuse and/or odorous discharge were the most frequent. Of the controls 80% did not have vaginal or vulvar complaints. In women with troublesome CCPD the duration of complaints was less than six months in nine (56%), from six months to two years in three (19%) and more than two years in four (25%).
Table II shows the main clinical and laboratory findings, bacteriological cultures not included, in patients and controls at first visit. While profuse discharge was infrequently noted in patients (7%) and in none of controls, discharge was significantly more often present in the vaginal vestibule of patients ($p=0.02$, Fisher's exact test). A frothy discharge was found in 43% of patients. The vaginal pH was ≤ 4.4 in all controls. Sixty percent of women with CCPD had a vaginal pH between 5.0 and 5.5. About half of the women with CCPD had at least a moderate number of leucocytes in the wet mount. Gas-liquid chromatography showed a succinate/lactate (S/L) ratio < 0.4 in all controls. Ninety-three percent of patients had a S/L ratio ≥ 0.4.
The microbiological results are shown in table III. *N.gonorrhoeae* was

Table III. Microbiological results at day 1 (controls and patients) and at day 9 and day 15 (patients)

		day 1 controls (n=10)	day 1 patients (n=30)	day 9 patients (n=30)	day 15 patients (n=30)
N.gonorrhoeae	+ve	0	1	((1))	0
ditto	–ve	10	29	28	29
ditto	n.d.	0	0	1	1
C.trachomatis	+ve	0	3	1((1))	0
ditto	–ve	10	27	26	29
ditto	n.d.	0	0	3((2))	1
T.vaginalis	+ve	0	0	1	0
ditto	–ve	10	29	28	28
ditto	n.d.	0	1	1	2
Candida species	+ve	2	4	9(7)	7(4)
ditto	–ve	8	24	19	22
ditto	n.d.	0	2	2((1))	1

n.d. = not done
( ) patient(s) in which preceding culture(s) was (were) -ve
(( ))patient(s) in which preceding culture(s) was (were) +ve

isolated two times, in one patient. She was treated with cefotaxime, one gramme i.m., and excluded from further study. Three patients had one or more positive chlamydial cultures. At day 1, *C. trachomatis* was isolated from three of 30 patients (10%). It was not isolated from controls. The difference is statistically not significant (p=0.56, Fisher's exact test). *T.vaginalis* was cultured once. The patient had to be regarded as an asymptomatic carrier. At the intake *Candida albicans* was cultured in two of 10 controls (20%) and in four of 30 patients (13%). This difference is not statistically significant (p=0.63, Fisher's exact test). The incidence of positive candidal cultures after treatment for CCPD is shown in table IV.

Treatment efficacy of the three different regimens is shown in figure 1. Improvement was observed in a considerable number of patients soon after starting treatment with amoxycillin alone or with amoxycillin plus clavulanic acid. Treatment with metronidazole resulted in the cure of four patients after only 24 hours. One to two days after completion of treatment (day 9), 'cure' was established in six patients from the amoxycillin group and in two from the amoxycillin-clavulanic acid group. At day 15 four women from the amoxycillin group and seven from the amoxycillin-clavulanic acid group had normal vaginal secretions. The use of metronidazole resulted in the cure of all patients by day 4, while in one patient treatment efficacy had to be described as 'indefinite' at day 15.

The vaginal pH was ≤ 4.4 in 17 of 20 cured patients (85%). It was > 4.4 in all five patients considered 'not cured'. In patients considered 'improved' and

Table IV. Results of candidal cultures* after treatment of CCPD with three different therapeutic regimens

	amoxycillin 500 mg t.i.d.		amoxycillin-clavulanic acid 625 mg t.i.d.		metronidazole 500 mg b.i.d.	
	day 9	day 15	day 9	day 15	day 9	day 15
Number of cultures taken	9	10	7	7	8	8
Culture result						
–	8	9	3	4	6	6
±	0	1	1	1	1	0
+	0	0	2	1	0	2
++	1	0	1	1	0	0
+++	0	0	0	0	1	0

* patients with positive cultures before treatment (n=4) not included

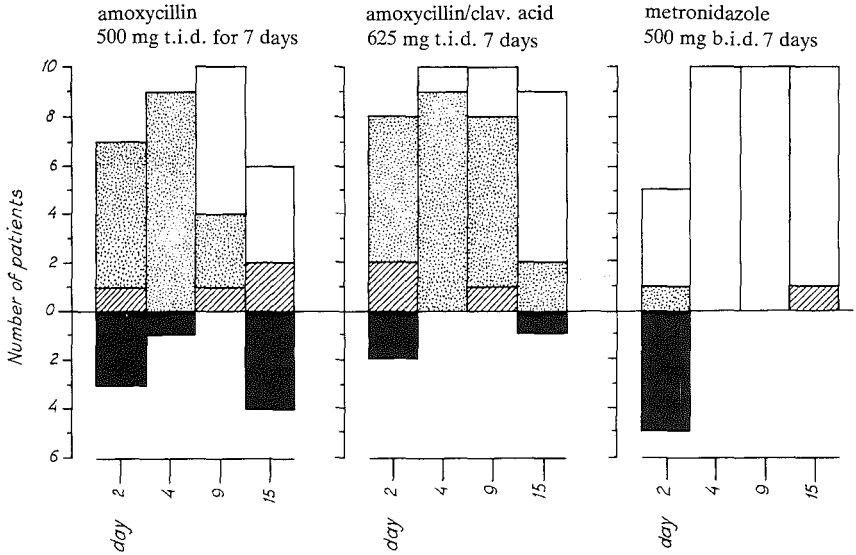


Figure 1 Results of treatment of CCPD with three different therapeutics at day 2, 4, 9 and 15 (□ cured, ▨ improved, ▨ indefinite, ■ not cured).

those labelled as 'indefinite', both normal (≤ 4.4) and increased pH values were measured. The S/L ratio was < 0.4 in 19 of 20 cured patients (95%) and in the two patients considered 'improved'. The S/L ratio was increased (≥ 0.40) in the three patients in whom treatment efficacy was labelled as 'indefinite', and in four of five patients in whom treatment had failed completely.

Only adverse effects volunteered by the patients were recorded. No complaints were recorded following the use of amoxycillin. In the amoxycillin-clavulanic acid group diarrhoea was mentioned by four of 10 patients (40%), but it subsided during treatment in all cases. Vulvar pruritus and/or burning, present in three women from this group, was due to overgrowth of *C.albicans*. In the metronidazole group two patients reported mild gastrointestinal symptoms.

Discussion

Our study indicates that CCPD is very often unnoticed by the women involved (table I). The fact that profuse discharge is a rather infrequent

Table V. Some clinical and laboratory findings in 30 patients with different treatment results at day 15

	cured (n=20)	improved (n=2)	indefinite (n=3)	not cured (n=5)
Amount of discharge				
scant	11	2	2	5
moderate	9	0	1	0
profuse	0	0	0	0
Odour	0	0	2	5
Positive amine test	0	0	1	5
Vaginal pH				
$\leqslant 4.4$	17	1	1	0
> 4.4	3	1	2	5
Wet mount				
clue cells	0	0	1	5
Döderlein's bacilli	20	0	0	0
Leucocytes (wet mount)				
none	1	0	0	1
few	11	1	2	1
moderate	5	1	0	1
many	3	0	1	2
S/L ratio				
< 0.40	19*	2	0	1
$\geqslant 0.40$	1	0	3	4**

* no succinate detectable in 8 cases
** no lactate detectable in 2 cases

finding is probably partly responsible.
The presence of discharge in the vaginal vestibule seems to be an important signal that 'something' is wrong. It probably does not discriminate between CCPD and trichomoniasis, the latter condition being characterized by profuse and watery secretions. The fact that 43% of the women with CCPD presented with a frothy discharge once again stresses that frothiness should not be considered pathognomonic for trichomoniasis.
The S/L ratio was $\geqslant 0.4$ in 28 of 30 patients (93%). This is in good agreement with other studies (Spiegel et al., 1980). In 7 of 28 samples (25%) lactate was not detected at all, probably because of the severely depressed state of the

lactobacillary flora. The view that anaerobic bacteria, within limited concentrations, constitute part of the normal vaginal flora is reflected by the detection of succinate in nine of 10 controls (90%).
We found no significant difference in the prevalence of cervical chlamydial infections between controls and patients at day 1. The higher isolation rate in patients could have something to do with the anaerobic environment in CCPD, which possibly favours colonization with *C. trachomatis*. The fact that there were only two patients with positive chlamydial cultures at day 1 in the amoxycillin and amoxycillin-clavulanic acid group does not permit statements about the anti-chlamydial activity of these preparations. However, since no positive cultures were found after treatment, further study on the anti-chlamydial activity of amoxycillin and amoxycillin-clavulanic acid seems warranted.
The relatively high prevalence (20%) of positive candidal cultures in asymptomatic women is generally agreed upon. The lower prevalence that we found in women with CCPD is probably explained by the increased vaginal pH in this condition, which impedes growth of vaginal yeasts. We observed a rather high incidence (57%) of positive candidal cultures at day 9 in women treated with amoxycillin-clavulanic acid (table IV). However, only one of these women was symptomatic. The occurrence of overgrowth of *Candida* species in the vaginal habitat after treatment with amoxycillin-clavulanic acid, is also commented upon by other authors (Pedler & Bint, 1985). *C. albicans* was cultured in two of 10 women (20%) treated with metronidazole. Our finding supports the view that candidal overgrowth is not a major sequela of metronidazole therapy.
Clinical cure and normalization of the S/L ratio (< 0.4) are closely correlated. The fact that succinate could not be detected in the secretions of eight of 19 cured women (42%), probably reflects the sub-physiological state of the anaerobic bacterial flora as a result of recent treatment. Normalization of the S/L ratio precedes clinical cure in a number of patients, especially in the amoxycillin and amoxycillin-clavulanic acid group (figure 2). Restoration of the lactobacillary flora to a microscopical detection level apparently takes longer with these regimens than with metronidazole.
So far as treatment efficacy is concerned, metronidazole must be considered a very potent drug. All patients had normal secretions at day 4 (figure 1). This possibly indicates that for example a three-day course is as effective as the standard seven-day regimen. Further comparative studies are, however, needed. Amoxycillin is capable of producing clinical improvement in the majority of patients soon after the start of treatment. Clinical cure, however, takes considerably longer. Furthermore, of six patients cured at day 9, two

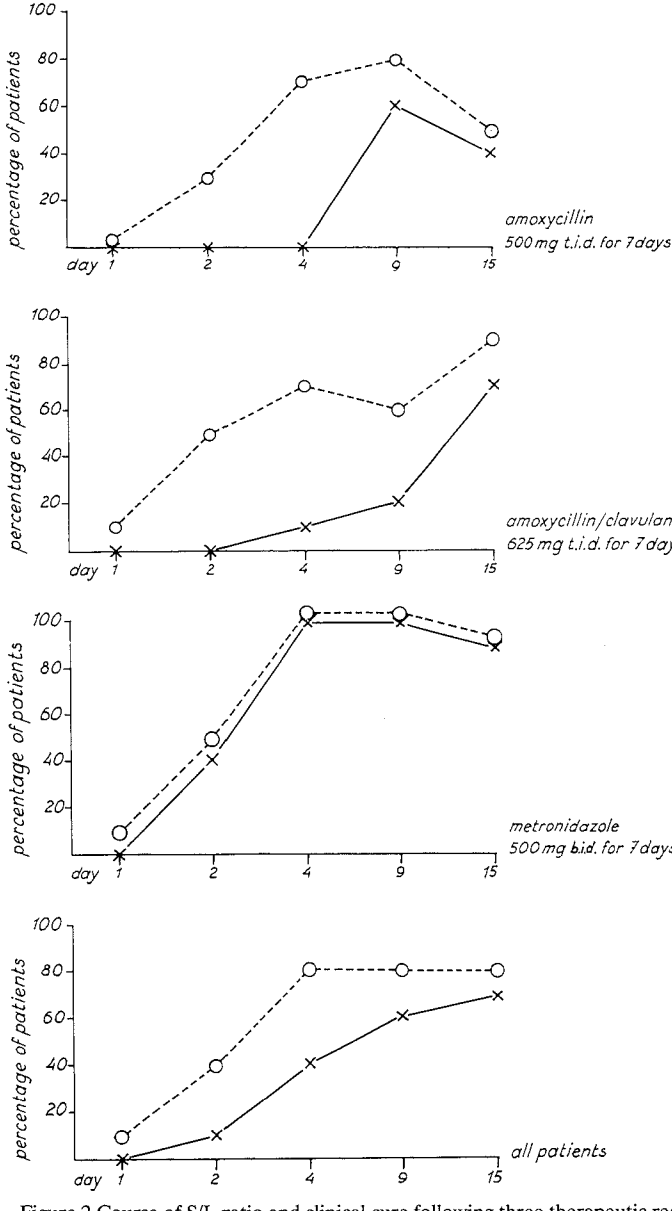


Figure 2 Course of S/L ratio and clinical cure following three therapeutic regimens (– – o – – o: S/L ratio <0.4; — x — x: clinical cure).

had a relapse at day 15. The relatively poor activity of penicillins against certain anaerobic bacteria, especially *Bacteroides* species (Hill & Ayers, 1985) could be the main reason.

Amoxycillin-clavulanic acid also produces considerable improvement shortly after the start of treatment. The cure rate at day 15 is higher than that of amoxycillin alone (70% versus 40%), probably because it has a better anti-anaerobic activity (Wüst & Wilkins, 1978). Whether or not the late reappearance of Döderlein's bacilli ($\geq$ day 9) is due to suppression by amoxycillin-clavulanic acid remains to be investigated.

In conclusion, we consider amoxycillin-clavulanic acid to be an acceptable alternative in the treatment of CCPD, although its efficacy seems somewhat lower and cure takes considerably longer than with metronidazole. In our opinion its use should be reserved for pregnant women and those showing metronidazole intolerance.

References

Hill, G.B. & Ayers, O.M. (1985). Antimicrobial susceptibilities of anaerobic bacteria isolated from female genital tract infections. Antimicrobial Agents and Chemotherapy 27, 324-31.

Høvik, P. (1983). Nonspecific vaginitis in an outpatient clinic. Comparison of three dosage regimens of metronidazole. Scandinavian Journal of Infectious Diseases suppl. 40, 107-10.

Jerve, F., Qvigstad, E. & Eng, J. (1983). Treatment of non-specific vaginitis with metronidazole. Scandinavian Journal of Infectious Diseases suppl. 40, 111-3.

Pedler, S.J., Bint, A.J. (1985). Comparative study of amoxycillin-clavulanic acid and cephalexin in the treatment of bacteriuria during pregnancy. Antimicrobial Agents and Chemotherapy 27, 508-10.

Pheifer, T.A., Forsyth, P.S., Durfee, M.A., Pollock, H.M. & Holmes, K.K. (1978). Nonspecific vaginitis: role of *Haemophilus vaginalis* and treatment with metronidazole. New England Journal of Medicine 298, 1429-34.

Piot, P., Van Dijck, E., Godts, P. & Vanderheyden, J. (1982). The vaginal microbial flora in non-specific vaginitis. European Journal of Clinical Microbiology 1, 301-6.

Piot, P. (1985). Bacterial vaginosis. An evaluation of treatment. Scandinavian Journal of Urology and Nephrology suppl. 86, 299-35.

Roe, F.J.C. (1983). Toxicologic evaluation of metronidazole with particular reference to carcinogenic, mutagenic, and teratogenic potential. Surgery 93, 158-64.

Spiegel, C.A., Amsel, R., Eschenbach, D., Schoenknecht, F. & Holmes, K.K. (1980). Anaerobic bacteria in nonspecific vaginitis. New England Journal of Medicine 303:601-7.

Van der Meijden, W.I. (1985). Clinical aspects of *Gardnerella vaginalis* - associated vaginitis. A review of the literature. Scandinavian Journal of Urology and Nephrology suppl. 86, 135-41.

Wüst, J. & Wilkins, T.D. (1978). Effect of clavulanic acid on anaerobic bacteria resistant to beta-lactam antibiotics. Antimicrobial Agents and Chemotherapy 13, 130-3.

IX TREATMENT OF CLUE CELL-POSITIVE DISCHARGE WITH 200 MG POVIDONE-IODINE PESSARIES. A DOUBLE-BLIND AND PLACEBO-CONTROLLED TRIAL*

Willem I. van der Meijden[1], Peter Piot [2], Paul I.M. Schmitz[3], Ernst Stolz[1]

Summary

In a double-blind randomized study we evaluated the efficacy of povidone-iodine in the treatment of clue cell-positive discharge (CCPD). Vaginal pessaries (200 mg povidone-iodine or placebo) were taken twice daily for five consecutive days. Although the regimen was reasonably well accepted, 'messiness' was reported by 13 of 33 women (39%). Of 44 women enrolled, treatment efficacy was evaluated in 28 women who had both follow-up visits. There was no significant difference in the efficacy of povidone-iodine and placebo pessaries, at both the first and second follow-up visit (p-values 0.46 and 1 respectively). It is concluded that the use of povidone-iodine pessaries, at least in the regimen described, cannot be regarded as an effective therapy of CCPD.

Introduction

Vaginitis is a very common gynaecological problem, with a tendency to recurrences on the one hand and chronicity on the other. It has therefore prompted a more or less continuous search for effective local and systemic treatment. This search has led to the development of several potent chemotherapeutics, some of which have been in use for a long time.
Trichomonal and bacterial vaginitis is generally treated by parenteral administration of drugs (e.g. metronidazole), while candidal infections are

* Eur J Obstet Gynecol Reprod Biol 1987; 24:299-307

[1] Department of Dermatology and Venereology, University Hospital 'Dijkzigt', Rotterdam, The Netherlands
[2] Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium
[3] Institute of Biostatistics, Erasmus University, Rotterdam, The Netherlands

most often locally treated (e.g. clotrimazole).
Povidone-iodine (polyvinylpyrrolidone-iodine, Betadine®, a well known skin disinfectant, has been used for local treatment of vaginitis since the early Sixties. It is claimed to have an antibacterial, antiprotozoal and antifungal effect in the vaginal habitat[1-4]. Its main advantage over iodine is that it possesses the antimicrobial activity of inorganic iodine but rarely causes irritation of skin and mucous membranes, even in iodine-sensitive individuals[5].
There is reason to believe that the vaginal use of povidone-iodine nowadays mainly concerns the treatment of chronic, non-specific vaginal infections, meaning vaginitis in the absence of *Trichomonas vaginalis*, *Candida* species and *Neisseria gonorrhoeae*. Most of these studies report moderate efficacy (up to 70%) of povidone-iodine, administered either as pessary, solution or gel[3,4,6,7]. To our knowledge, only one study seriously questioned the efficacy of povidone-iodine as a vaginal therapeutic. In this study it was concluded that povidone-iodine did not have a pronounced beneficial effect as compared with a placebo. However, spontaneous cure was reported in more than half the cases[8].
Our study was undertaken to investigate the efficacy of povidone-iodine in the treatment of bacterial vaginosis (*Gardnerella vaginalis*-associated vaginitis), a condition recently designated as 'clue cell-positive discharge'[9]. This specific disease entity is characterized by homogeneous and malodorous vaginal discharge, showing clue cells in the wet mount. *Gardnerella vaginalis* and certain obligate anaerobic bacteria play a major role in its pathogenesis[10].

Materials and methods

Study population. Patients were recruited from women who visited the outpatient department of venereology, in order to be checked for sexually transmitted diseases. They were enrolled if the wet mount of their vaginal secretions showed clue cells in the absence of *T.vaginalis* and pseudohyphae. Women with multiple sexual partners (e.g. prostitutes), and those who had used antibiotics within the preceding two weeks, were excluded.
Treatment. The treatment of patients with clue cell-positive discharge (CCPD) was double-blind and placebo-controlled. The vehicle in both the povidone-iodine and placebo pessaries consisted of polyethyleneglycol (Hoechst). The placebo pessaries contained Opalake CL-9304 (Colorcon) as a pigment.
Treatment consisted of the deposition of one pessary (200 mg povidone-

iodine or placebo) deep in the vagina in the morning and the evening of five consecutive days, starting the day after the first consultation.
Monitoring. During first visit (intake) and both follow-up visits (1-2 days after completion of therapy and one week later) anamnestic data, as well as several clinical parameters were recorded. At each session a small sample of vaginal discharge was suspended in saline and deep-frozen (–20°C), in order to do a gas-chromatographic analysis on non-volatile organic acids (succinate, lactate) in due time. Gas-liquid chromatography was performed as described elsewhere[11]. Cultures for *N. gonorrhoeae* (cervix, urethra and rectum), *Chlamydia trachomatis* (cervix), *T. vaginalis* (posterior fornix) and *Candida* species (lateral vaginal wall) were taken at all three visits.
In monitoring treatment efficacy, a patient was considered 'improved' when there was non-odorous discharge and a negative amine test, while the wet mount showed neither clue cells nor Döderlein's bacilli. The reappearance of a bacillary flora was supposed to indicate 'cure'. The conclusion 'not improved' indicates persistance or reappearance of the 'fullblown' picture of CCPD.

Results

Table I shows some characteristics of the women who were enrolled. The mean age of the total study group was 30.3 years (SD=7.7). The contraceptive behaviour of the povidone-iodine and the placebo group did not differ significantly.
Of the women enrolled, only 23 (52%) had troublesome discharge, the most frequent complaints being profuse discharge and/or offensive odour. None of the women reported vulval itching or irritation. The duration of complaints was less than one month in 10 and from 1-6 months in 7 women. Six

Table I. Some characteristics of women with CCPD who were enrolled in the povidone-iodine study

	povidone-iodine (n=19)	placebo (n=25)
Mean age ± SD	29.9 ± 7.0 yr	30.6 ± 8.3 yr
Contraception		
none	11(58)	15(60)
pill	5(26)	7(28)
IUD	2(11)	3(12)
Condom	1(5)	0(0)

Table II. Main characteristics of vaginal discharge in women with CCPD (intake)

	patients (n=44)
Amount	
scant	20(45)
moderate	22(50)
profuse	2(5)
Colour	
white	24(55)
whitish-yellow	17(39)
yellowish-white	2(5)
other (e.g.blood-stained)	1(2)
Frothiness	
present	16(36)
absent	28(64)
Offensive odour	
present	44(100)
absent	0(0)
Amine test	
positive	43(98)
negative	0(0)
doubtful	1(2)
Vaginal pH	
<4.5	4(9)
4.5-5.0	2(5)
5.0-5.5	20(45)
5.5-6.0	17(39)
>6.0	1(2)
Leucocytes (wet mount)	
none	7(16)
few	19(43)
moderate	15(34)
many	3(7)
S/L ratio	
≥0.4	37(84)*
<0.4	4(9)**
n.d.	3(7)

( ) number in parentheses = percentage
* no lactate detectable in 12 samples
** no succinate detectable in 1 sample
n.d. = not done

women had had troublesome discharge for a longer period. The main characteristics of the vaginal discharge (at intake) are shown in Table II. It indicates that profuse vaginal discharge is a rather uncommon finding in

patients with CCPD. Thirty-six percent of the women had a frothy discharge, while offensive odour was present in all. The amine test, in which the secretions on the lower valve of the speculum are mixed with some KOH 10%, was positive in 43 of 44 cases (98%). Vaginal secretions had a mean pH of 5.3 (SD=0.5). Gas chromatographic analysis of 41 vaginal samples showed a succinate/lactate ratio (S/L ratio) ≥0.4 in 37 cases (90%). In 12 of 41 samples lactate was not detected at all.

Table III shows the association of gas chromatographic and clinical findings in patients treated for CCPD (first follow-up visit). A S/L ratio ≥0.4 is significantly more often associated with offensive odour, positive amine test and presence of clue cells, than is a S/L ratio <0.4 ($p<0.001$). The microbiological results are shown in Table IV. There were two positive gonococcal cultures, both from the same patient (placebo group). This patient was withdrawn from the study at the second follow-up visit. *Chlamydia trachomatis* was isolated 10 times in 6 different patients (Table

Table III. Succinate/lactate ratio (S/L ratio)and clinical findings in patients treated for CCPD, at first follow-up visit *

	S/L ratio ≥0.4 (n=12)	S/L ratio <0.4 (n=20)	p-value**
Odorous discharge	11(92)	6(30)	
Non-odorous discharge	1(8)	14(70)	p<0.001
Amine test positive	11(92)	5(25)	
Amine test negative	1(8)	15(75)	p<0.001
pH >4.5	9(75)	11(55)	
pH ≤4.5	0(0)	5(25)	p=0.12
pH 'not measurable'	3(25)	4(20)	
Clue cells present	9(75)	4(20)	
Clue cells absent	1(8)	15(75)	p<0.001
Clue cells 'doubtful'	2(17)	1(5)	
Döderlein's bacilli absent	11(92)	11(55)	
Döderlein's bacilli present	1(8)	6(30)	p=0.19
Döderlein's bacilli 'doubtful'	0(0)	3(15)	

* gas chromatography was not performed in one case

( ) number in parentheses = percentage

** Fisher test. Findings reported as 'not measurable' or 'doubtful' were not incorporated into the statistical test

Table IV. Microbiological results at intake (a) and first (b) and second (c) follow-up visit

culture		povidone-iodine a (n=19)	b (n=15)	c (n=17)	placebo a (n=25)	b (n=18)	c (n=15)
N.gonorrhoeae	+ ve	0	0	0	1	1	0
ditto	–ve	19	15	17	24	15	15
ditto	n.d.	0	0	0	0	2	0
C.trachomatis	+ ve	1	1	2	2	2	2
ditto	–ve	18	13	14	23	14	12
ditto	n.d.	0	1	1	0	2	1
T.vaginalis	+ ve	0	0	0	0	0	1
ditto	–ve	19	15	17	25	16	14
ditto	n.d.	0	0	0	0	2	0
candida species	+ ve	0	0	0	6	5	3
ditto	–ve	17	15	16	19	10	11
ditto	n.d.	2	0	1	0	3	1

n.d. = not done

Table V. Isolation of *C.trachomatis* in six patients, before and after treatment for CCPD

patient number	a	b	c	
1	–	+	–	povidone-iodine
2	+	–	+	
3	–	–	+	
4	+	+	+	placebo
5	–	+	+	
6	+	–	–)	

a = intake
b = first follow-up visit
c = second follow-up visit

V). *Trichomonas vaginalis* was isolated once. The patient had malodorous discharge, the wet mount of which did show clue cells but no trichomonads. Six patients, all from the placebo group, had a positive yeast culture at the first examination. Growth was indicated as sporadic or grade 1 in all cases. Altogether, yeast cultures were positive 14 times in 9 different patients. In

only one patient were all three cultures positive.
The acceptability of the treatment regimen was investigated in 33 women who had at least a first follow-up visit (Table VI). Overall, 76% of the women found the regimen acceptable, although 5 of 25 women considered 'messiness' a disadvantage. There was no significant difference in the acceptability of povidone-iodine and of placebo pessaries (p=0.24). The 2 women from the povidone-iodine group who disliked the pessaries considered 'vaginal dryness' and 'unpleasant feeling' the main objection. The 6 women from the placebo group all reported unacceptable 'messiness'.
As far as treatment efficacy is concerned, only women who had both follow-up visits were taken into account. Table VII shows that there is no significant difference in the efficacy of povidone-iodine and placebo pessaries, at both the first and the second follow-up visit (p-values 0.46 and 1 respectively). Moreover, the 3 women from the povidone-iodine group who were considered 'cured' at the first follow-up visit, all had a recurrence one week later.

Discussion

The above described study indicates that profuse vaginal discharge is an uncommon finding in patients with CCPD (Table II). This could be the main reason why about half of the women with this condition did not report troublesome discharge. The fact that this disease entity is rarely, if ever, accompanied by inflammation of the vaginal wall (Kaufman RH, Gardner HL, unpublished data, 1965), is probably also responsible. In an earlier study, however, it was shown that upon direct questioning the majority of

Table VI. The acceptability of vaginal pessaries in the treatment of CCPD

patient's judgement	povidone-iodine (n=15)	placebo (n=18)	
acceptable	13(87)	12(67)	
not acceptable	2(13)	6(33)	p=0.24**
oral treatment preferred	3(20)	5(29)	*
oral treatment not preferred	7(47)	9(53)	*
no preference at all	5(33)	3(18)	*

( ) number in parentheses = percentage
* preference of one patient unknown
** Fisher test

Table VII. Results of treatment for CCPD, one to two days after the end of therapy (1st follow-up visit) and one week thereafter (2nd follow-up visit)

	povidone-iodine (n=15)*	placebo (n=13)*
First follow-up visit		
not improved	8(53)	9(69)
improved	4(27)	2(15)
cured	3(20)	2(15) p=0.46**
Second follow-up visit		
not improved	12(80)	11(85)
improved	0(0)	0(0)
cured	0(0)	2(15)
recurrence	3(20)	0(0) p=1**

( ) number in parentheses = percentage
* concerns only patients who had both follow-up visits
** Fisher test: 'not improved' versus other categories

women were aware of vaginal discharge and especially offensive odour[11]. Gas chromatographic analysis of 41 samples of women with CCPD showed a S/L ratio ≥ 0.4 in 37 cases (90%). This finding is in good agreement with the studies of Spiegel et al. and Hill, who found 89% and 96% respectively[10,12]. There is a significant difference between vaginal discharge with a S/L ratio ≥ 0.4 and discharge with a ratio <0.4, as far as the presence of vaginal offensive odour, positive amine test and clue cells is concerned ($p<0.001$). The height of vaginal pH and the absence or presence of Döderlein's bacilli seem less discriminative (p-values 0.12 and 0.19 respectively). It is postulated that normalization of the S/L ratio precedes the reappearance of a bacillary flora. Further study on this subject seems warranted.

The evidence from the literature that povidone-iodine possesses a trichomonacidal and yeast-killing activity is inconclusive[2,4,13,14]. The results of our study do not permit conclusions in this respect, the number of positive cultures being too small (Table IV). The prevalence of chlamydial infections in the women studied could not be calculated exactly. In the six women with one or more positive cultures, culture positivity was a rather inconsistent finding (Table V). The prevalence, however, seems lower than other data suggest [15].

The treatment regimen was reasonably well accepted (Table VI). The main reason for disliking treatment was 'messiness', especially in the women treated with placebo. More 'messiness' in the placebo group, illustrated by

the fact that all cases of pH 'not measurable' (brownish redisue) were in the latter (Table III), possibly reflects slower absorption and/or evacuation from the vagina of the placebo pigment than of povidone-iodine.
While some investigators advocate the use of povidone-iodine in the treatment of 'non-specific' vaginal infections, our study does not support the idea that povidone-iodine has a 'broad spectrum antimicrobial activity' in the vaginal habitat, at least not in the regimen that we used. Most authors reporting good efficacy of povidone-iodine in the treatment of vaginal infections, advocate its use for a prolonged period, up to 3 weeks. It could therefore be that longer treatment results in a higher cure rate. However, Dattani et al. [8], treating patients with 200 mg Betadine® pessaries twice daily for 2 weeks, did not report good efficacy with such a regimen. Their observation that spontaneous cure occurred in over half the cases, could not be confirmed (Table VII). Since effective oral treatment for CCPD is available, i.e. metronidazole, 500 mg b.i.d. for one week [16], alternative therapies should not exceed 5-7 days, in order to ensure patient compliance. The observation of one of us (vdM), that CCPD tends to persist in prostitutes who practice daily vaginal irrigation with a povidone-iodine solution, suggests that one should not expect less treatment failures with such a regimen.

References

1. Shook DM. A clinical study of a povidone-iodine regimen for resistant vaginitis. Curr Ther Res 1963; 5: 256-263.
2. Ratzan JJ. Monilial and trichomonal vaginitis. Topical treatment with povidone-iodine preparations. Calif Med 1969; 110: 24-27.
3. Harris PG. Clinical experience with povidone-iodine vaginal pessaries in the treatment of non-specific vaginitis. Practitioner 1972; 209: 828-830.
4. Hauser GA. Neues, die Döderleinflora schonendes Vaginal- desinfiziens zur Therapie unspezifischer Vaginitiden. Schweiz Rundschau Med (Praxis) 1975; 64: 1289-1294.
5. Shelanski HA, Shelanski MV. PVP-iodine: history, toxicity and therapeutic uses. J Int Coll Surg 1956; 25: 727-734.
6. Singha HSK. The use of a vaginal cleansing kit in non-specific vaginitis. Practitioner 1979; 223: 403-404.
7. Rhemrev PER. The use of Betadine microbicides in the treatment of nonspecific vaginitis. Proceedings World Congress on Antisepsis, Limburg/Lahn, W.Germany, 1976; 63-64.
8. Dattani IM, Gerken A, Evans BA. Aetiology and management of non- specific vaginitis. Br J Vener Dis 1982; 58: 32-35.
9. Van der Meijden WI. Clinical aspects of *Gardnerella vaginalis*- associated vaginitis. A review of the literature. Scand J Urol Nephrol 1985; suppl 86: 135-141.
10. Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK. Anaerobic bacteria in nonspecific vaginitis. New Engl J Med 1980; 303: 601-607.

11. Piot P, van Dijck E, Godts P, Vanderheijden J. The vaginal microbial flora in non-specific vaginitis. Eur J Clin Microbiol 1982; 1: 301-306.
12. Van der Meijden WI. Treatment of non-specific vaginitis with a single dose of tinidazole. Scand J Infect Dis 1983; suppl 40: 85- 89.
13. Hill LVH. Anaerobes and *Gardnerella vaginalis* in non-specific vaginitis. Genitourin Med 1985; 61: 114-119.
14. Saggers BA, Stewart GT. Polyvinylpyrrolidone-iodine: an assessment of antibacterial activity. J Hyg 1964; 62: 509-518.
15. Duran AA, Laris PL. Clinical experience with Betadine microbicides in the treatment of vaginitis of mixed etiology. Proceedings World Congress on Antisepsis, Limburg/Lahn, W.Germany, 1976; 65-68.
16. Stamm WE, Holmes KK. *Chlamydia trachomatis* infections of the adult. In: Sexually Transmitted Diseases (ed. Holmes KK, Mårdh P- A, Sparling PF, Wiesner PJ), p 259-260. McGraw-Hill Book Company, 1984.
17. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Non- specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. New Engl J Med 1978; 298: 1429-1434.

X GENERAL DISCUSSION

This thesis studied the extent of the problem of vaginal discharge, from the doctors' and the patients' point of view, as well as some diagnostic, pathogenetic and therapeutic aspects.

Our epidemiological study showed that abnormal vaginal discharge is a common finding among women visiting the Rotterdam sexually transmitted diseases (STD) clinic. Clue cell-positive discharge (bacterial vaginosis, non-specific vaginitis) is the most prevalent vaginal disease entity, a finding also reported from gynaecological departments[1].
So far as diagnosis is concerned, we confirmed that symptoms and signs are not of much help in predicting specific vaginal infections, due to the considerable overlap between the different entities. Smelling the secretions on the withdrawn speculum has to be considered a major diagnostic tool, since it is an inexpensive and very effective way to discriminate between *probably* normal (non-odorous) and *certainly* abnormal (malodorous) secretions. Therefore, it is a procedure with which every clinician dealing with gynaecological problems should become familiar.
The search for features discriminating between bacterial vaginosis and trichomoniasis was only moderately successful. Since both conditions are characterized by malodorous discharge, one could expect the results of anaerobic cultures and gas-liquid chromatography to be more or less identical. It is evident that an infection with *Trichomonas vaginalis* relatively often leads to vaginitis, which is reflected by the presence of parabasal cells in a considerable number of patients. The finding that symptoms and signs were more often mild in the case of a combined presence of *T. vaginalis* and clue cells, than in the case of trichomoniasis alone, needs further elucidation.
Microscopy of the wet mount should be performed in all cases of malodorous secretions, in order to find out whether it is clue cell-positive discharge (CCPD), or trichomoniasis, or both, and in the case of non-odorous secre-

tions with a peculiar aspect (curdy) and/or 'specific' complaints (pruritus/ dysuria) in a search for pseudo-hyphae. Since microscopy is only moderately sensitive, especially in the case of a vaginal yeast infection, additional pathogen-detecting methods will have to be used in those cases in which a (strong) suspicion of a vaginal infection is not supported by the result of microscopy. So far, culture is the only readily available alternative. However, it takes considerable time before the results are known. We hopefully await (the development of) tests that can be read in the office (e.g.agglutination tests) and, above all, that provide the clinician with *quantitative* information.
In some patients with vaginal and vulvar complaints one cannot find a causative agent despite a thorough and repeated search. Psychosomatic vulvovaginitis is said to be a real clinical entity, which should be suspected in any patient whose vaginal complaints do not correlate with the physical findings[2]. However, this condition is beyond the scope of this thesis.
The survey in general practices revealed that troublesome discharge is common, especially in younger women. Both the complaint and the need for information on the subject were strongly related to the age of the respondents, steadily decreasing with growing age. Since research data show that many women regard vaginal discharge as 'something normal', information should primarily focus on such questions as 'what is normal discharge?' and 'when should I seek medical advice about vaginal discharge?'. The general practitioner seems the most suitable person to give this information, provided that he is well-informed himself. Since many women still have to be persuaded that vaginal discharge is not 'fate' but merely an incovenience, often amenable to treatment, much information will also have to be provided to a general public via general channels, e.g. women's magazines.

It is generally agreed upon that bacterial adherence to epithelial cells is a prerequisite for colonization and, eventually, bacterial infection. Clue cells can be considered a very fine example of bacterial adherence *in vivo*. The great adhering capacity of *Gardnerella vaginalis*, an important constituent of the bacterial flora in CCPD, has been repeatedly shown[3]. In CCPD only a minority of vaginal epithelial cells (VECs) are covered with bacteria. This 'selective' bacterial adherence seems to depend on vitality and glycogen content of the VECs. Both are significantly decreased in the case of CCPD, probably as a result of anaerobic overgrowth. It is hypothesized that the VECs in CCPD go through several stages: 1. preadherence, vital, much glycogen 2. adherence, vital, less glycogen 3. adherence, non-vital, no glycogen and 4. postadherence, non-vital, no glycogen. Since it is evident

that bacterial adherence is a major process in the pathogenesis of CCPD, further study of bacterium-VEC interaction in the vaginal habitat is necessary, especially because it could have therapeutic implications. The study of bacterial adherence during treatment could help to understand why a certain product is effective and another is not.

Since in general CCPD is a clinically mild disease, treatment is perhaps best restricted to symptomatic women. Third-trimester pregnancy possibly also needs consideration in view of the increased risk of preterm labour[4]. The introduction of metronidazole for this condition, in the very effective and almost 'classic' regimen of 2x500 mg for one week[5], has to be considered a break-through. However, since metronidazole is sometimes not tolerated (antabuse effect) and should only reluctantly be used during pregnancy, a continuous search for other regimens and especially for alternative treatments has been going on.
We studied the efficacy of single-dose treatment with tinidazole (another nitroimidazole derivative), the therapeutic range of amoxycillin plus clavulanic acid (a broad-spectrum penicillin with good anaerobicidal properties), as well as the effect of local povidone-iodine. Some studies reporting good efficacy of single-dose nitroimidazole treatment had only a short follow-up, thus overlooking the significance of relapses. Amoxycillin-clavulanic acid appeared reasonably effective in the treatment of CCPD. It is much more effective than amoxycillin alone, probably because of its better anti-anaerobic activity[6]. Re-establishment of the normal flora (Döderlein's bacilli), however, takes considerably longer than with the use of metronidazole. Its use should be reserved for pregnant women and those showing metronidazole intolerance. Due to inaccessible niches, local treatment of vaginal infections is often disappointing. This could be the main reason for the observed inefficacy of povidone-iodine. A longer treatment period may give better results but cannot be advocated in view of negative effects on patient compliance.
In conclusion, since abnormal vaginal discharge, especially CCPD, is rather prevalent among women of childbearing age, it is very important that doctors involved should have adequate knowledge of diagnostic and therapeutic possibilities, of which an overview has been presented. There is still much to be learned about women's attitudes towards vaginal discharge, and especially about the major reasons of patient delay.

References

1. Fleury FJ. Adult vaginitis. Clin Obstet Gynecol 1981; 24: 407-438
2. Dodson MG, Friedrich EG. Psychosomatic vulvovaginitis. Obstet Gynecol 1978; 51: 23S-25S.
3. Mårdh P-A, Weström L. Adherence of bacteria to vaginal epithelial cells. Infect Immun 1976; 13: 661-666.
4. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986; 67: 229-237.
5. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. New Engl J Med 1978; 298: 1429-1434.
6. Wüst J, Wilkins TD. Effect of clavulanic acid on anaerobic bacteria resistant to beta-lactam antibiotics. Antimicrob Agents Chemother 1978; 13: 130-133.

XI SUMMARY

This thesis discusses several aspects of (abnormal) vaginal discharge, focusing especially on clue cell-positive discharge (bacterial vaginosis, non-specific vaginitis).

Chapter I introduces the study subject and highlights some major results of international research.
Chapter II formulates the study objectives.

Chapters III and IV present clinical and laboratory data on specific vaginal infections, especially clue cell-positive discharge (CCPD) and vaginal trichomoniasis. The latter two entities show a considerable overlap in clinical and laboratory findings. Both are characterized by anaerobic overgrowth. Trichomoniasis relatively often leads to inflammation of the vaginal wall, which is reflected by the presence of parabasal cells in the wet mount. Without stating that registration of symptoms and signs should be omitted, it is obvious that smelling the vaginal secretions provides the clinician with more useful information. It easily discriminates between *probably* normal (non-odorous) and *certainly* abnormal (malodorous) secretions.
Chapter V provides data from women visiting general practices. Troublesome vaginal discharge is frequently present, especially among younger women. Vaginal discharge is often regarded as 'something normal'. Only a minority of women therefore consult their general practitioner. Information on vaginal discharge was considered desirable by 61% of the respondents, and should preferably be supplied by general practitioners, women's magazines and television.

Chapter VI shows that in the case of CCPD the percentages of vital and glycogen-containing vaginal epithelial cells (VECs) are considerably lower than in normal secretions, which is probably due to anaerobic overgrowth.

This could account for the relatively low percentage of clue cells in the VEC population of CCPD.

Chapters VII through IX report on the efficacy of different treatment regimens. Treatment with a single dose (2 grammes) of tinidazole showed an efficacy of only 50% and is therefore not propagated. Although restoration of a normal bacillary flora takes considerably longer with amoxycillin-clavulanic acid (3x625 mg for one week) than with metronidazole (2x500 mg for one week), the former compound seems a reasonable alternative in the treatment of CCPD, especially in pregnant women and in the case of metronidazole intolerance. Povidone-iodine pessaries (200 mg), inserted twice daily for 5 days, were not effective.

Chapter X discusses the major study results in the context of data from the literature.

SAMENVATTING

In dit proefschrift worden een aantal aspecten van (abnormale) vaginale afscheiding aan de orde gesteld, waarbij de aandacht speciaal is gericht op 'clue cell-positive discharge' (bacteriële vaginose, niet-specifieke vaginitis).

Hoofdstuk I leidt het onderwerp in en belicht enkele belangrijke, internationale onderzoeksresultaten.
Hoofdstuk II beschrijft het voornaamste doel van de verschillende studies.

De hoofdstukken III en IV beschrijven de resultaten van klinisch en laboratoriumonderzoek bij vrouwen met specifieke vaginale infecties, in het bijzonder 'clue cell-positive discharge' (CCPD) en vaginale trichomoniasis. De laatste twee entiteiten vertonen veel overeenkomst, zowel wat de kenmerken van de fluor zelf betreft, als betreffende laboratorium resultaten. Ze worden beide gekwalificeerd door een overmaat aan anaërobe bacteriën. Trichomoniasis gaat relatief vaak gepaard met een ontsteking van de vaginawand, hetgeen wordt weerspiegeld door de aanwezigheid van parabasale epitheelcellen in het direkte microscopische preparaat. Zonder te willen beweren dat van het opnemen van anamnese en het noteren van klinische verschijnselen moet worden afgezien, zal duidelijk zijn dat het vaststellen van de geur van de vaginale afscheiding de medicus practicus meer bruikbare gegevens oplevert. Het is op deze wijze eenvoudig, onderscheid te maken tussen *waarschijnlijk* normale (reukloze) en *zeker* abnormale (riekende) afscheiding.
In hoofdstuk V wordt verslag gedaan van een enquête onder vrouwen die hun huisarts bezochten. Hieruit blijkt, dat hinderlijke vaginale afscheiding vaak voorkomt, met name bij jongere vrouwen. Vaginale afscheiding wordt vaak beschouwd als 'iets normaals'. Een minderheid van de geënqueteerden zou met 'afscheiding' naar de huisarts gaan. Een-en-zestig procent van de respondenten wenste informatie over dit onderwerp. Deze zou bij voorkeur verstrekt moeten worden door huisarts, damesbladen en televisie.

In hoofdstuk VI wordt aangetoond dat de percentages vitale en glygogeenbevattende cellen in de celpopulatie van CCPD aanzienlijk lager zijn dan die in normale afscheiding, hetgeen waarschijnlijk een gevolg is van de overmaat aan anaërobe bacteriën. Dit zou het relatief kleine percentage 'clue cells' in de celpopulatie van CCPD kunnen verklaren.

De hoofdstukken VII t/m IX behandelen de effectiviteit van diverse therapeutische regimes. Aangezien een eenmalige gift van 2 gram tinidazol slechts in 50% der gevallen effect sorteerde, wordt deze behandeling niet aanbevolen. Alhoewel het herstel van een normale bacillaire flora bij gebruik van amoxycilline-clavulaanzuur (3x625 mg gedurende 1 week) aanzienlijk langer duurt dan bij gebruik van metronidazol (2x500 mg gedurende 1 week), lijkt eerstgenoemde verbinding een redelijk alternatief bij de behandeling van CCPD, met name bij zwangeren en in geval van het niet kunnen verdragen van metronidazol. Het gedurende 5 dagen tweemaal per dag inbrengen van 200 mg povidon-jodium zetpillen was niet effectief.

Hoofdstuk X bespreekt de belangrijkste onderzoeksresultaten, in het kader van gegevens uit de literatuur.

APPENDIX

photos 1- 4: examination by speculum, original magnification 6x

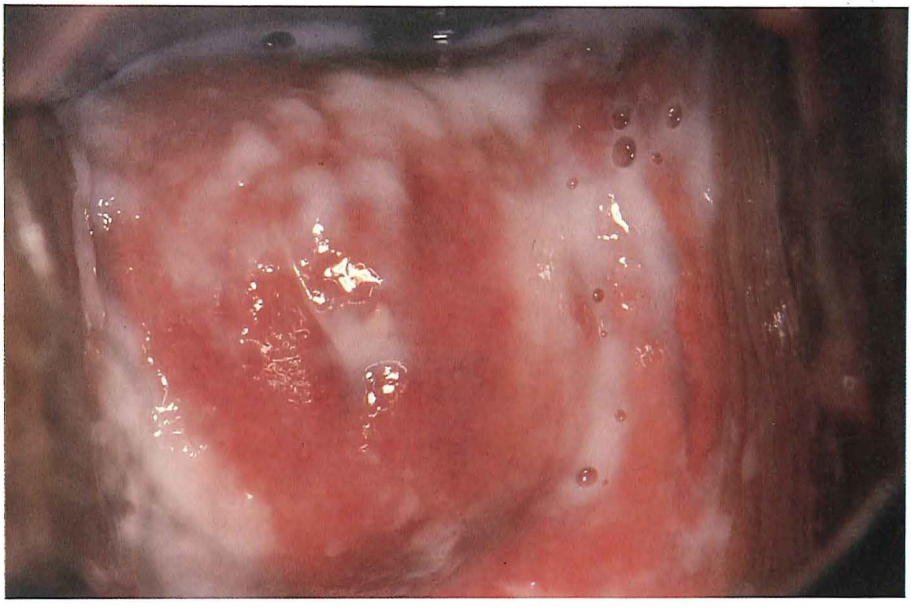


photo 1 : normal secretions

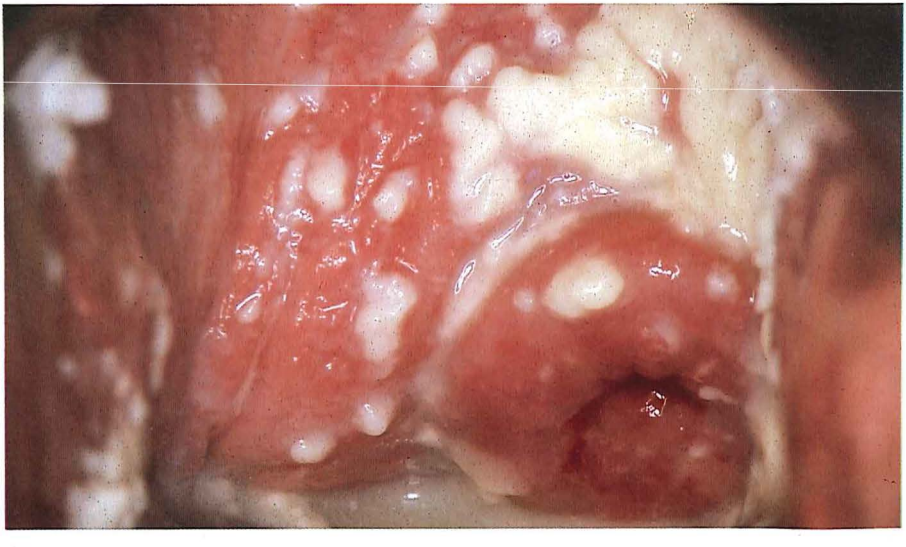


photo 2 : vaginal candidosis

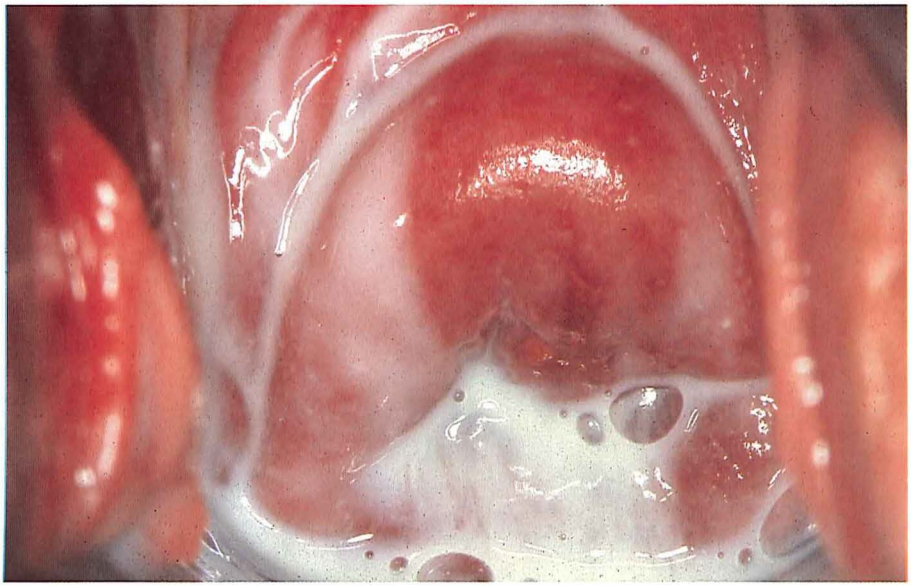


photo 3 : bacterial vaginosis

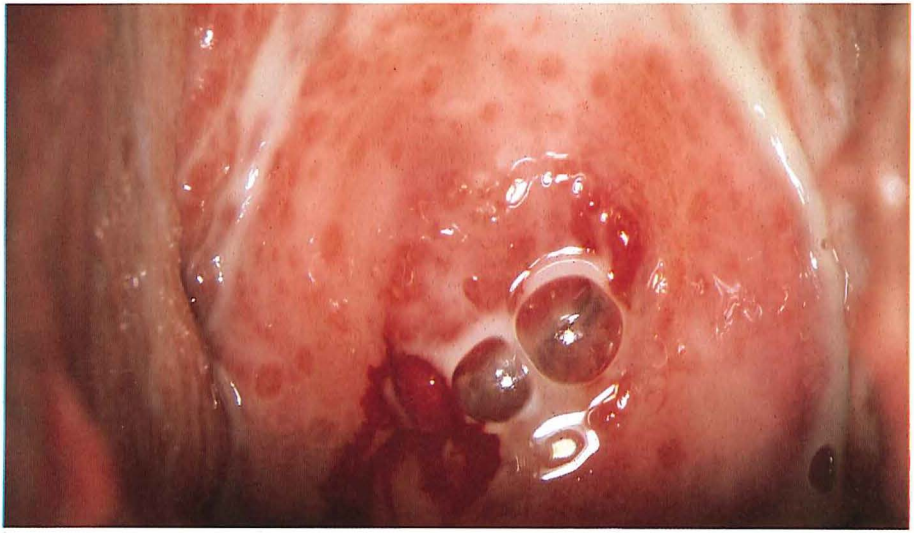


photo 4 : vaginal trichomoniasis ('strawberry' cervix)

photos 5- 8: wet mount preparations, original magnification 400x

photo 5 : normal secretions (Döderlein's bacilli)

photo 6 : vaginal candidosis (pseudo-hyphae)

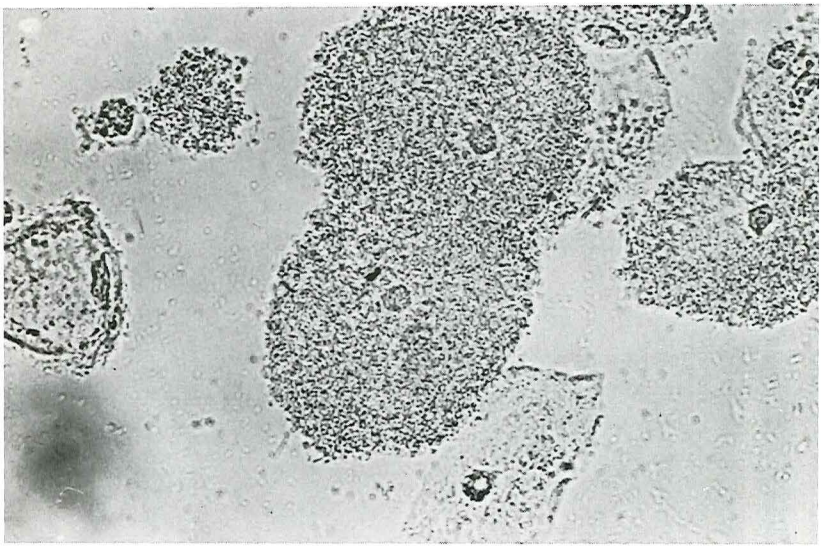


photo 7 : clue cell-positive discharge (clue cells)

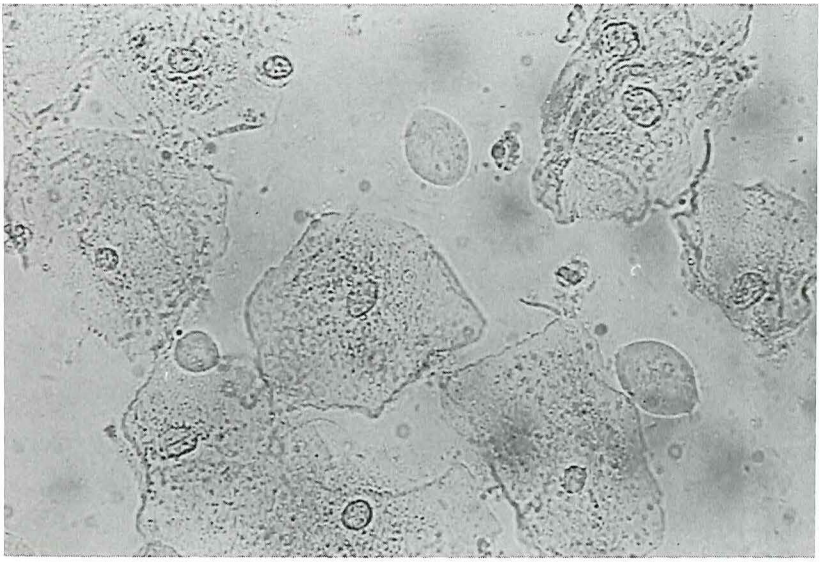


photo 8 : vaginal trichomoniasis (trichomonads)

photos 9-12: Gram-stained preparations, original magnification 400x

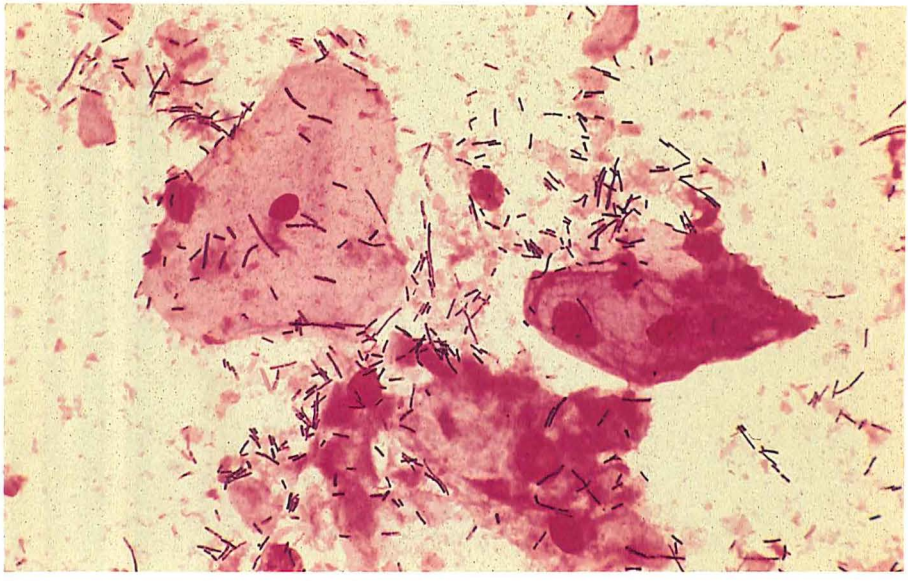


photo 9 : normal secretions (Gram-positive bacilli)

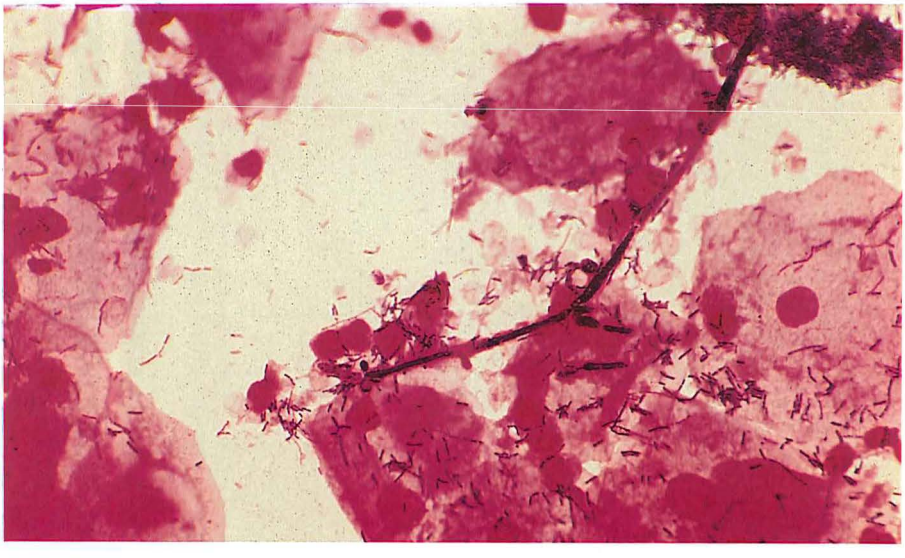


photo 10 : vaginal candidosis (Gram-positive pseudo-hyphae and bacilli)

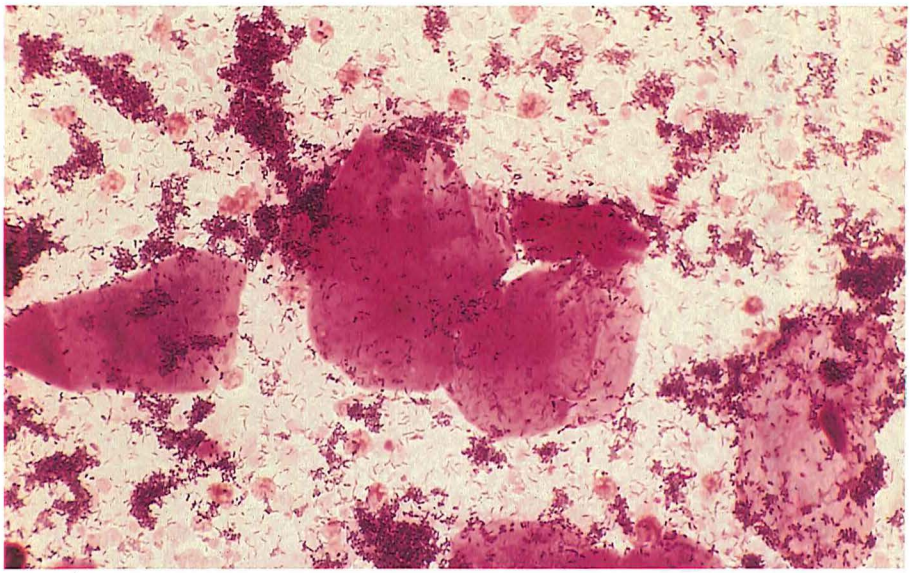


photo 11 : clue cell-positive discharge (Gram-negative curved rods)

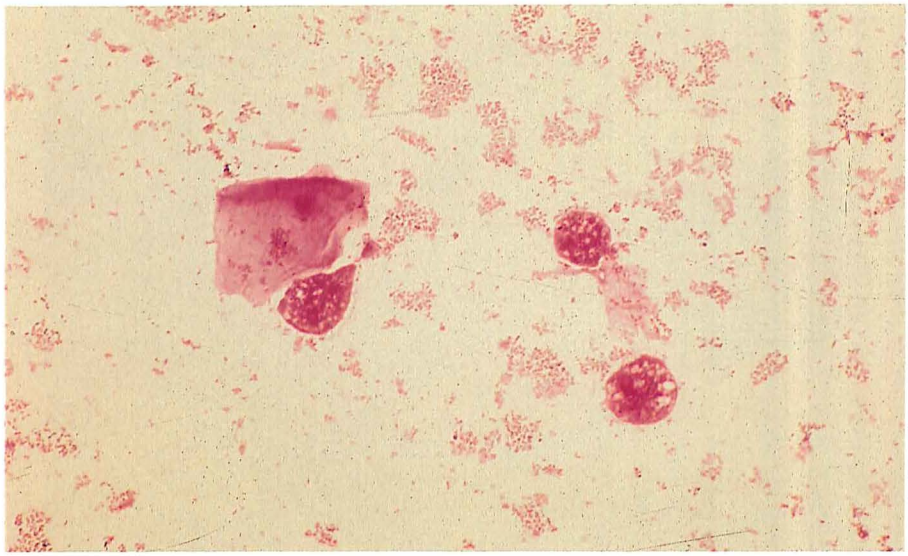


photo 12 : vaginal trichomoniasis

photos 13-14: vital staining (trypan blue 1%), original magnification 200x

photo 13 : normal secretions

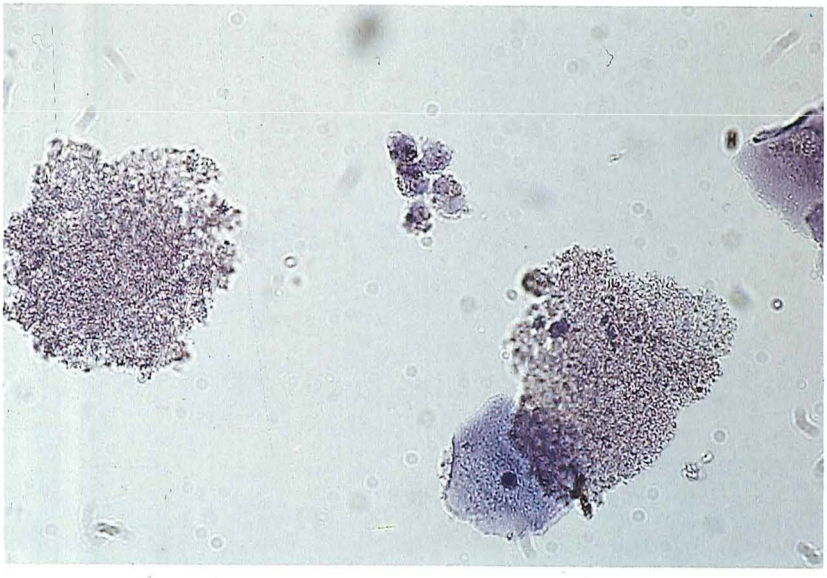


photo 14 : clue cell-positive discharge

photo 15-16: glycogen staining (PAS), original magnification 200x

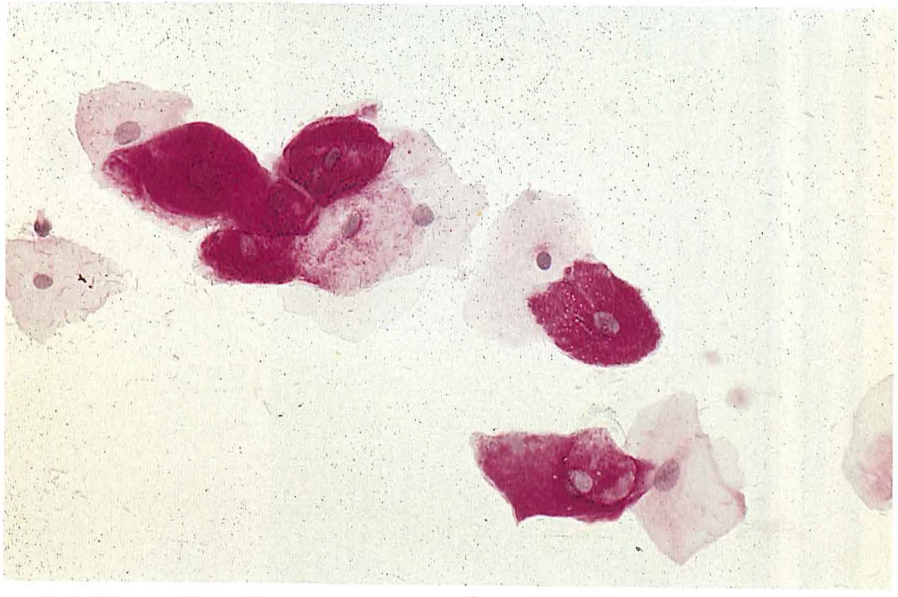


photo 15 : normal secretions

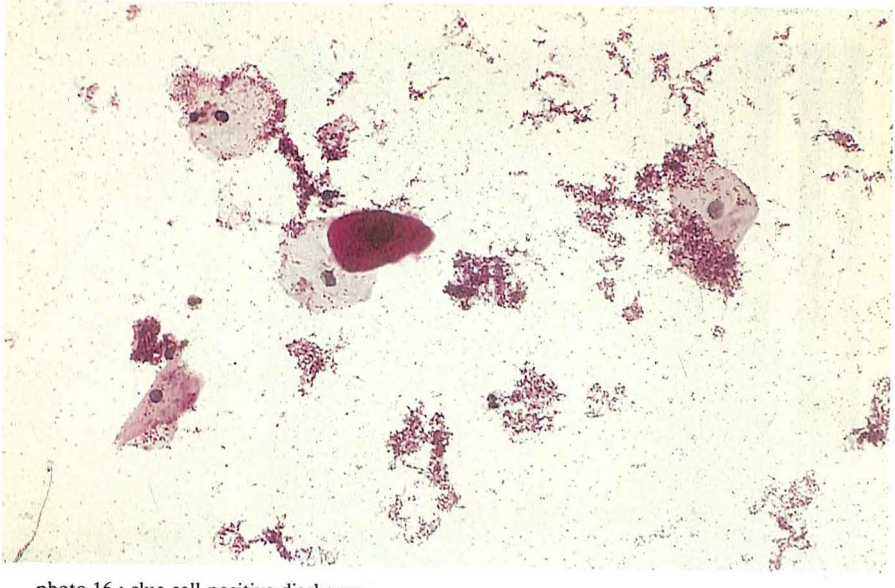
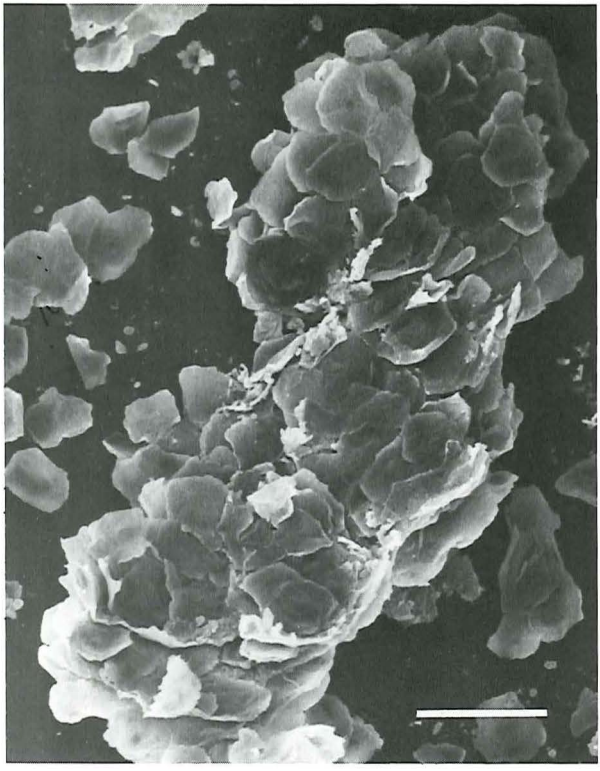


photo 16 : clue cell-positive discharge



photos 17-20: scanning electron micrographs

photo 17 : normal secretions, cluster of vaginal epithelial cells (VECs), bar = 100 µm.

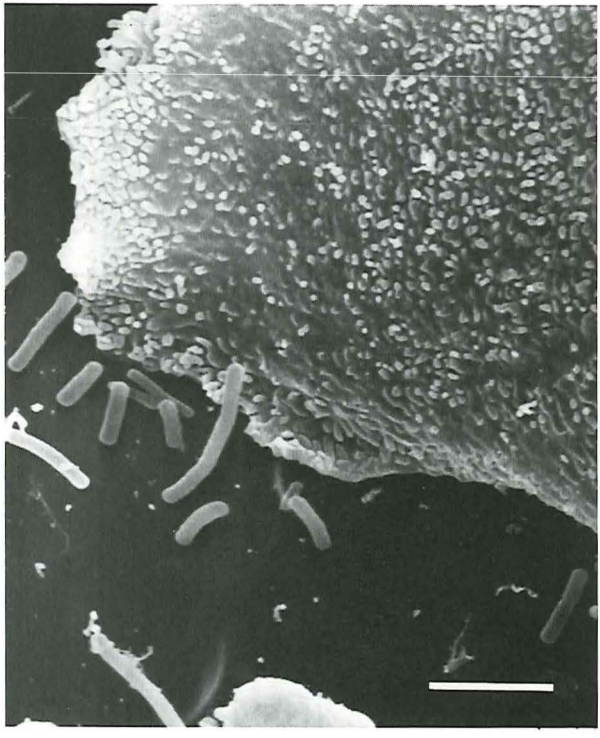


photo 18 : normal VEC and Döderlein's bacilli, bar = 5 µm

photo 19 : clue cell with adjacent normal VEC, bar = 5 μm

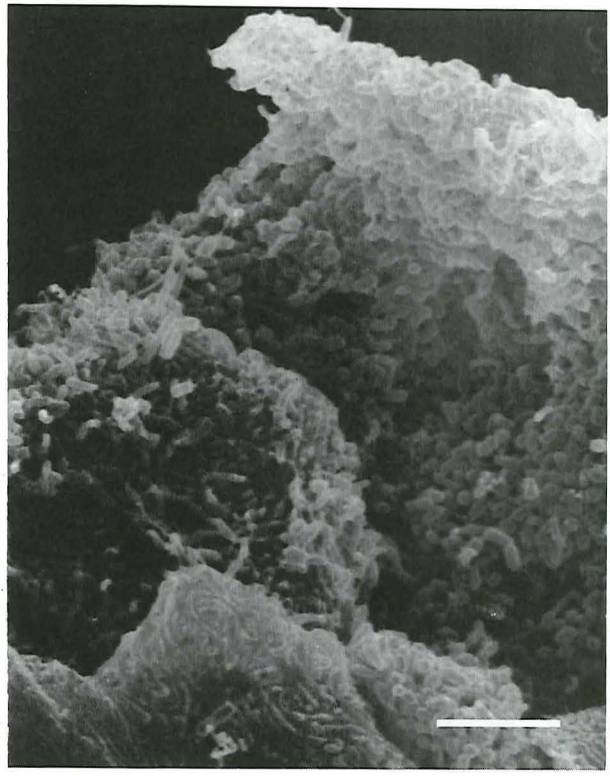
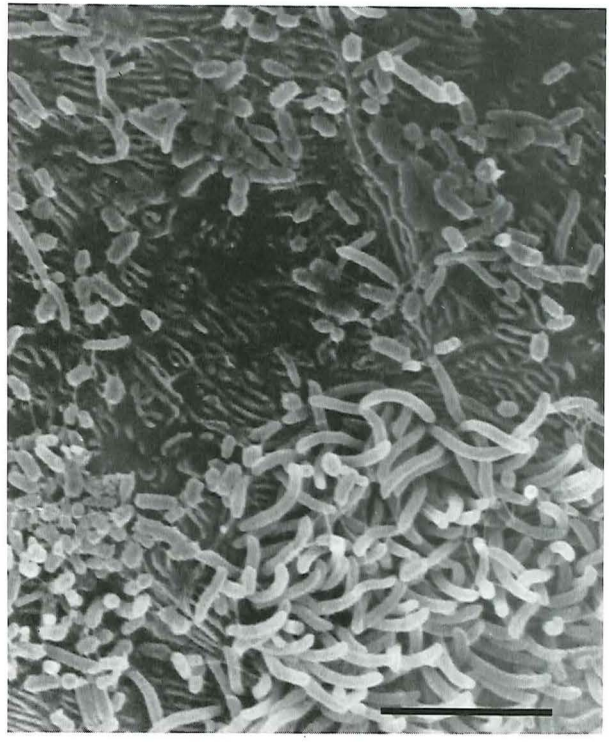
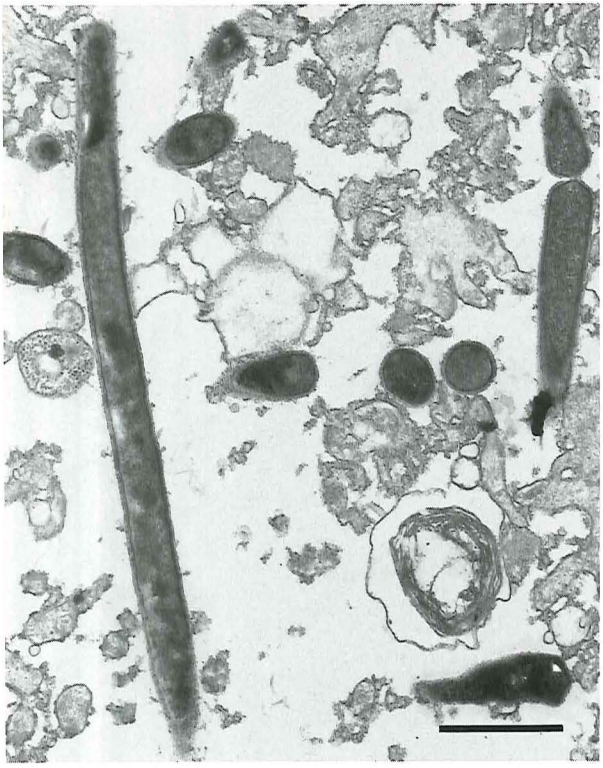


photo 20 : curved rods attached to VEC, bar = 5 μm.





photos 21-24: transmission electron micrographs

photo 21 : Doderlein's bacilli alongside VEC, bar = 1 μm

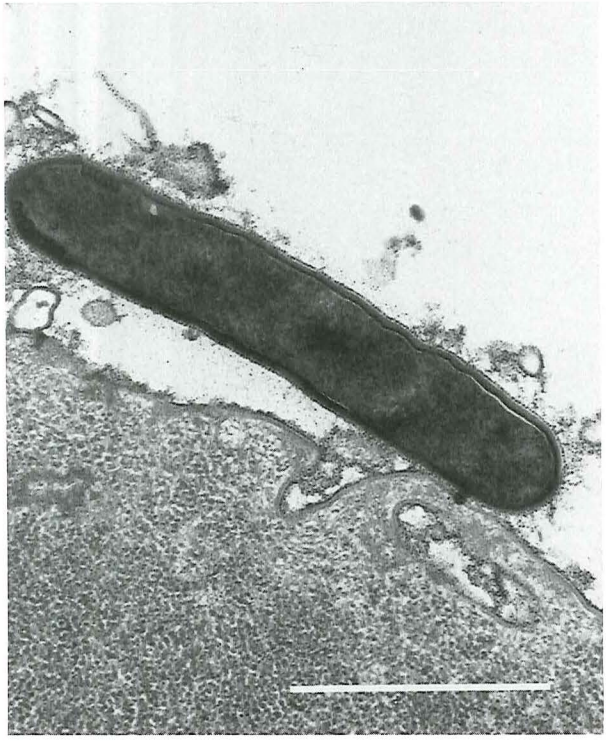


photo 22 : Döderlein's bacillus on VEC surface, bar = 1 μm

photo 23 : clue cell, bar = 5 µm

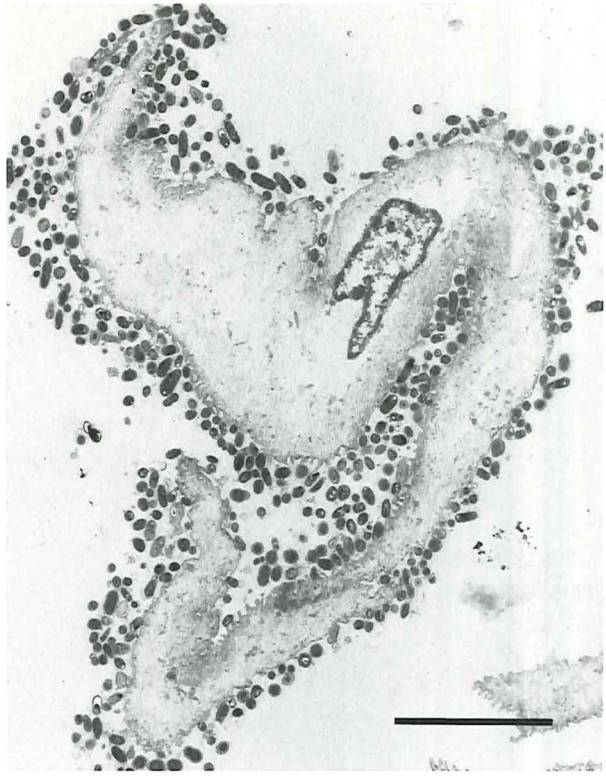
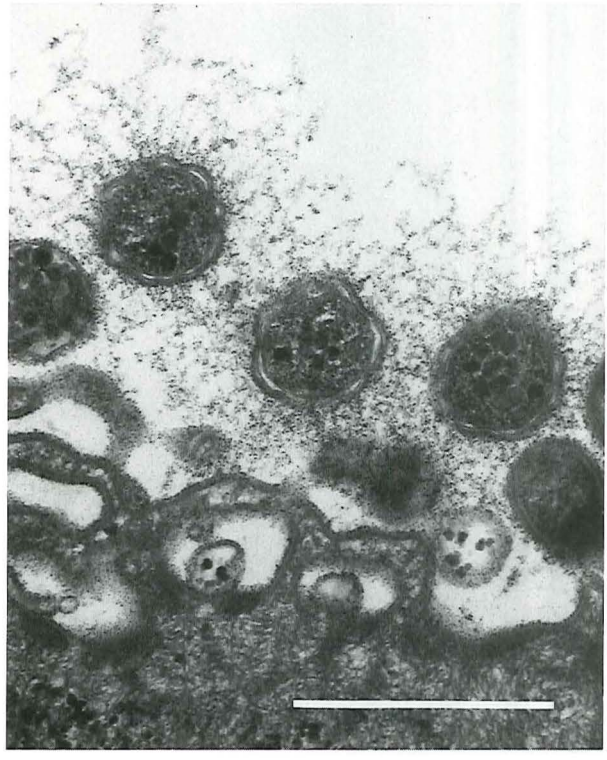


photo 24 : bacterial glycocalyces, bar = 1 µm



photos 25-28: negatively stained vaginal secretions (phosphotungstic acid 2%)

photo 25 : Döderlein's bacilli, bar = 1 µm

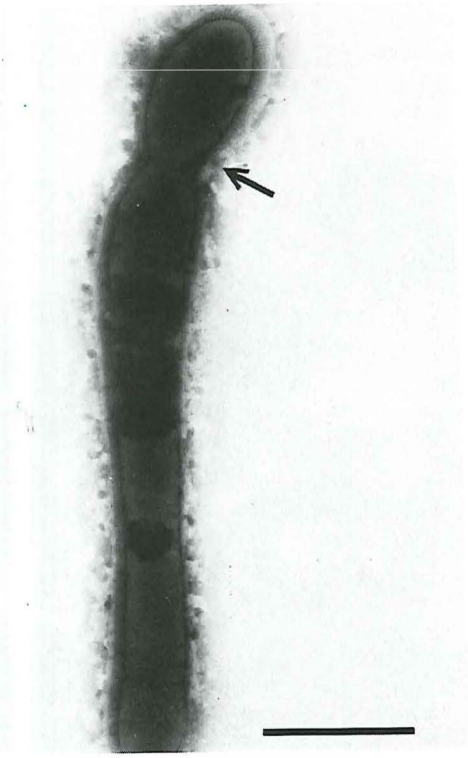


photo 26 : Döderlein's bacillus(septum formation, see arrow),
bar = 1 µm

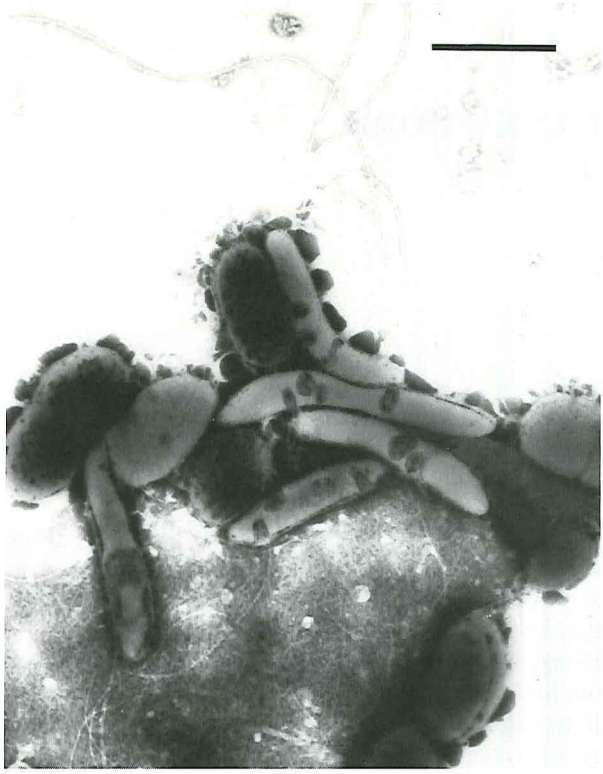


photo 27 : flagellated curved rods and cocci on VEC surface, bar = 1 μm

photo 28 : flagellated curved rod, bar = 1 μm

DANKWOORD

Aan allen die, op welke wijze dan ook, hebben meegewerkt aan de totstandkoming van dit proefschrift wil ik mijn hartelijke dank betuigen. Enkelen wil ik speciaal noemen.
Hooggeleerde Drogendijk, beste Aat. Jij bent er de afgelopen jaren, mede dankzij jouw grote interesse in het deelgebied der gynaecologische infecties, steeds weer in geslaagd mij als onderzoeker in het juiste spoor te houden. Ik ben je hiervoor zeer erkentelijk.
Hooggeleerde Stolz, beste Ernst. Hartelijk dank voor de gelegenheid die je me hebt geboden om een belangrijk deel van het promotieonderzoek op jouw afdeling uit te voeren. Ik waardeer het bovenal, dat jij het enkele jaren geleden aandurfde een ander specialisme binnen de afdeling dermato-venereologie te introduceren.
Hooggeleerde Piot, beste Peter. Jouw deskundigheid op het gebied van bacteriële vaginose en de prettige wijze waarop ik daarmee, bij herhaling, werd geconfronteerd, mogen niet onvermeld blijven. Ik hoop dat ook in de toekomst met het Instituut 'Prins Leopold' kan worden samengewerkt.
Zeergeleerde De Bruijn, beste Wim. Op geheel eigen wijze introduceerde je mij in het veld der electronenmicroscopie. Dat er een wereld voor me openging, heb ik aan jou te danken.
Zeergeleerde Duivenvoorden en Schmitz, beste Hugo en Paul. De enthousiaste en vriendschappelijke wijze waarop jullie de statistische bewerkingen voor jullie rekening namen, zal ik niet licht vergeten.
Zeergeleerde De Haes, beste Willy. Onze gezamenlijke aktiviteiten op het gebied van gezondheidsvoorlichting en -opvoeding heb ik als zeer positief en leerzaam ervaren.
Johan van der Stek was mede verantwoordelijk voor de voortreffelijke kwaliteit van de zwart-wit en meerkleuren-illustraties. Zijn '24-uurs-service' dient in ere gehouden te worden.
Hans Moes ben ik dankbaar voor de wijze waarop hij mij steeds weer de weg wees in de administratieve 'jungle', hetgeen kostbare tijd spaarde.

Mijn collegae en de verpleegkundigen van de polikliniek dermato-venereologie dank ik voor de grote belangstelling waarmee de voortgang van het onderzoek werd gevolgd. Het werd als zeer motiverend ervaren.
Paula Vogelaar-Looy heeft bij de voltooiing van het proefschrift een belangrijke rol gespeeld. Haar snelle en accurate wijze van 'tekstverwerking' waren van onschatbare waarde.
Emile Loof dank ik voor de prettige samenwerking. Zijn vooruitziende blik heeft in belangrijke mate bijgedragen tot de realisering van dit proefschrift.
Lieve Heleen, Ido en Walter. Dat wij als gezin 'de eindstreep hebben gehaald', stemt mij tot grote dankbaarheid. Jullie adviezen en commentaar hebben mij geholpen te beseffen dat de realiteit van een promovendus beperkt is.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 5 september 1949 te Brielle geboren. Hij doorliep aldaar de middelbare school (H.B.S.-B).
Van september 1966-october 1973 studeerde hij medicijnen aan de Vrije Universiteit te Amsterdam. Aansluitend vervulde hij de militaire dienstplicht (Koninklijke Marine), grotendeels als officier-arts aan boord van Harer Majesteits 'Van Speijk'.
Na enkele maanden in een tweetal huisarts-praktijken te hebben waargenomen, werd in november 1975 begonnen met een wisselassistentschap chirurgie/gynaecologie -obstetrie in het St. Elisabeth's Gasthuis te Arnhem. De specialisatie gynaecologie-obstetrie werd op 1 april 1977 aangevangen in het ziekenhuis 'De Weezenlanden' te Zwolle (hoofd: dr. G.J.W.H. Lenters). De opleiding werd vervolgd en afgesloten in het St. Franciscus Gasthuis te Rotterdam (hoofd: dr. H.P.C.M. Hoynck van Papendrecht). Op 1 april 1982 vond registratie als vrouwenarts plaats.
Van 1 april 1982 tot 1 april 1983 was de auteur werkzaam als medisch adviseur van Rhône-Poulenc Nederland B.V., in welke periode hij zich, met name, intensief met huisartsen-nascholing heeft beziggehouden.
Sedert 1 april 1983 is de auteur staflid van de afdeling dermato-venereologie van het Academisch Ziekenhuis 'Dijkzigt' te Rotterdam, alwaar een belangrijk deel van het promotie-onderzoek werd uitgevoerd.