Prospective Analysis of Genital Ulcer Disease in Brooklyn, New York

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We prospectively studied 82 men and women with first episodes of genital ulceration. By using newer diagnostic techniques, a definite microbial etiology of 84 infections in 65 of the 82 patients evaluated was found. There were 33 cases of definite primary syphilis, 27 of definite chancroid, and 24 of definite genital herpes simplex. Conclusive evidence of more than one microbial etiology was found for 19 (23%) of the patients. Simultaneous primary syphilis and chancroid was the third most common ulcer infection. This finding underscores the need for both clinical suspicion of multiple infections in patients with genital ulcers and comprehensive testing for all suspicious etiologies.

During the past decade, prospective studies on genital ulcer disease have been largely confined to tropical populations. African studies have confirmed that *Haemophilus ducreyi* is a common pathogen [1–6] and have ominously suggested that genital ulcers increase the risk of transmission and acquisition of HIV infection [7, 8].

In a previous prospective study published in 1978 [9], herpes simplex virus was the leading cause of genital ulcer disease in an urban clinic in the United States. During the 1970s and early 1980s, syphilis was largely a disease of homosexual men [10], and except for localized outbreaks, chancroid was rare in the United States [11–13].

The epidemic of HIV infection led to changes in sexual behavior that resulted in a decreased incidence of syphilis in homosexual men. The incidence of early syphilis in the United States, however, increased during the late 1980s. This rise was associated with the use of crack cocaine, occurred largely among urban minority heterosexual populations, and generated a concomitant increase in the incidence of congenital syphilis [10]. In certain urban centers, the increased incidence of early syphilis was accompanied by an increased incidence of genital ulcer disease due to other etiologies as well [14]. Between 1986 and 1988 in New York City, the number of reported cases of chancroid, early syphilis, and genital ulcers due to herpes simplex virus increased from 556 to 1,140 (105%), 589 to 1,165 (98%), and 324 to 577 (78%), respectively [15].

The last decade has also brought improved diagnostic tools for evaluating genital ulcers. Direct fluorescent antibody (DFA) stains have facilitated the rapid diagnosis of genital herpes [16–18] and syphilis [19]. Improved culture techniques for the evaluation of *H. ducreyi* have led to the isolation of the organism in 61%–81% of presumptive cases [20]. This study was undertaken to prospectively determine the etiology of genital ulcer disease in Brooklyn, New York, by using contemporary diagnostic techniques.

**Materials and Methods**

**Study Population**

The study was conducted at the Sexually Transmitted Disease Clinic of the Kings County Hospital Center in Brooklyn from 13 September 1988 to 31 May 1990. Patients with genital ulcer disease who presented to the clinic when an investigator was present were asked to participate in the study. A genital ulcer was defined as a lesion on the surface of the skin of the penis or labia with loss of superficial tissue. There were no minimum requirements for width or depth. Patients were eligible for the study if they had not received topical or oral antimicrobials within the past week or if they did not have a history of genital ulcers or genital vesicles.

All patients underwent a standard interview, physical examination, and laboratory evaluation. Illicit drug use, prostitution, frequency of condom use, sexual preference, history of sexually transmitted diseases, number of different sexual partners in the preceding month, and duration of genital ulceration before seeking medical care were recorded. The number of ulcers, their location, and the presence of pain, induration, purulence, and inguinal adenopathy were noted. Patients were treated on the basis of the results of the initial evaluation and were asked to return for a follow-up visit 1 week later.

**Laboratory Evaluation**

Exudate from the ulcer base was inoculated onto two media and examined for *H. ducreyi*. Briefly, a sterile cotton swab was rolled in the ulcer base and plated onto Mueller-Hinton agar (BBL Becton Dickinson Microbiology Systems, Cockeysville, MD) with 5% chocolate horse blood agar (BBL Becton Dickinson Microbiology Systems), 1% IsoVialEx (BBL Becton
methods at the initial visit. All patients received counseling mal antibody absorption (FTA-ABS) test, according to routine concerning HIV infection and were offered voluntary confi-

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were collected and examined by darkfield microscopy for spiro­

hours, and transported to the laboratory. Finally, ulcer exudates

were placed in chlamydia transport media, stored at 4°C for 1- 2

hours and transported to the laboratory. Specimens for cultures for herpes simplex virus were ob­
tained by scraping the ulcer base with a cotton swab and placing it in 2 mL of viral transport media. Specimens were stored at 4°C for 1-2 hours and transported to the laboratory. Specimens for the isolation of Chlamydia trachomatis were obtained by using flexible wire calcium alginate swabs (Spectrum Labora­
tories, Houston). Samples taken from the ulcer base were placed in chlamydia transport media, stored at 4°C for 1-2 hours, and transported to the laboratory. Finally, ulcer exudates were collected and examined by darkfield microscopy for spirochetes.

Plates for cultures for H. ducreyi were incubated in a moist candle extinction jar at 36°C for 48 hours and then examined daily for 1 week before discarding. The presumptive identifica­tion of H. ducreyi was based on typical colonial morphology (small yellow-gray colonies that could be moved intact across the agar surface) and gram staining demonstrating gram-negative bacilli with a typical “school of fish” or “railroad track” pattern. The identification was confirmed by oxidase positivity
determined with use of tetramethyl-p-phenylenediamine dihy­
drocyanide reagent (Becton Dickinson, Mountain View, CA) and demonstration of a requirement for X factor (BBL Becton Dickinson Microbiology Systems) but not V factor (BBL Becton Dickinson Microbiology Systems) on GC agar containing 0.1% D-glucose (Merck, Rahway, NJ), 0.01% L-glutamine (Sigma, St. Louis), and 0.05% L-cysteine (J. T. Baker, Phil­lipsburg, NJ).

Specimens for cultures for herpes simplex virus were inocu­lated onto an A549 cell line and incubated at 37°C for 2 weeks. Identification of herpes simplex virus was based on the typical cytopathic effect. Specimens for cultures for C. trachomatis were inoculated onto cycloheximide-treated McCoy cells and incubated at 36°C for 65-70 hours. Immunofluorescence (Sa­nofi-Pasteur, Chaska, MN) for typical inclusions was per­formed at 48 hours.

All patients underwent serological tests for syphilis, includ­ing a rapid plasma reagin (RPR) test and a fluorescent trepone­mal antibody absorption (FTA-ABS) test, according to routine methods at the initial visit. All patients received counseling concerning HIV infection and were offered voluntary confi-

dential testing for antibody to HIV. Testing for antibody to HIV was performed by the New York City Department of Health. Both a positive ELISA and a confirmatory western blot established HIV positivity.

Results

Epidemiology

During the study period, as previously reported, 35% of visits to our sexually transmitted disease clinic were because of genital ulcer disease [21]. An investigator was present during 50% of the clinic sessions. Forty-seven women and 35 men who met study criteria (first episode of genital ulcer disease and no recent antimicrobial use) sought care while an investiga­tor was present in the clinic and were enrolled in the study. Information on the number of patients excluded and the reasons for exclusion was not collected.

The ages of the women and men ranged from 17 to 47 years and 17 to 74 years, respectively. A history of a nonulcerative sexually transmitted disease was reported by 14 women (30%) and 13 men (37%). Forty-five women (96%) and 27 men (77%) reported infrequent or no condom use during sexual intercourse. At the time of study enrollment, eight (17%) of the 47 women were pregnant; all were in their third trimester, and all but one had untreated syphilis.

Twenty-seven patients (33%) reported the use of crack co­caine. A significant difference was noted between men and women: women (21 [45%]) were more likely than men (six [17%]) to be crack cocaine users (P < .01; OR, 3.66; CI, 1.36–11.1). Men reported giving money or drugs for sex (15 [43%]) more frequently than women acknowledged receiving money or drugs for sex (eight [17%]) (P < .01; OR, 3.66; CI, 1.33–10.1).

One man and one woman reported intravenous drug use; one man acknowledged homosexual activity. These three pa­tients were infected with HIV. Sixty-six other patients with no identifiable risk for HIV infection other than a history of unprotected heterosexual intercourse agreed to testing for antibodies to HIV. In all, five (13%) of the 40 women and three (12%) of the 26 men were infected with HIV. Six women and seven men declined testing for antibodies to HIV.

Thus, of 69 patients who agreed to testing, 11 (16%) were HIV-positive; unprotected heterosexual intercourse was the presumed mode of transmission in eight (73%) of these pa­tients. This rate is lower than the previously reported HIV positivity rate of 26% among all patients with genital ulcer disease in our clinic [21], which is most probably due to our exclusion of patients with recurrent disease in this study. Frequent crack cocaine use was reported by six (75%) of eight HIV-positive patients with no identifiable risk for HIV infection.
Microbiology

At least one microbial etiology of the genital ulcer(s) was identified definitely for 65 (79%) of the 82 patients. Multiple etiologies were conclusively identified for 19 patients (23%). No definite diagnosis could be made for 17 (21%) of the 82 patients studied, yet presumptive evidence of at least one microbial etiology was found for 14 patients (17%) (tables 1–3).

As shown in table 1, evidence of *H. ducreyi* was found in the genital ulcer(s) of 40 patients (49%). *H. ducreyi* was isolated from the ulcers of 27 patients (definite chancroid). Gram staining was also positive for 24 (89%) of these patients. Culture alone was positive for three patients (11%). *H. ducreyi* was successfully isolated with use of both media (MH-HB agar and GC-HgS agar) in 20 cases (positive gram staining, 18; negative gram staining, two). In five cases, *H. ducreyi* was isolated on MH-HB agar alone, and in two cases, the organism was isolated on GC-HgS agar alone. Finally, gram staining of ulcer exudates from 13 (33%) of the 40 patients were suggestive of *H. ducreyi*, but cultures of these specimens were negative (presumptive chancroid).

Evidence of primary syphilis was found in the genital ulcers of 38 (46%) of the 82 patients (table 2). A definite diagnosis of primary syphilis was made for 33 patients (40%), and a presumptive diagnosis was made for an additional five patients (6%). A definite diagnosis was defined as either positive darkfield microscopy or a positive DFA test with other supporting laboratory evidence (positive RPR or FTA-ABS test) or concomitantly positive darkfield microscopy and DFA test.

Of note, four patients who presented 2 to 3 days after the onset of their ulcers and for whom serological tests were negative but darkfield microscopy and a DFA test were positive were classified as having definite primary syphilis. Eight patients had positive serologies with negative darkfield microscopy, a negative DFA test, and no history of syphilis. Two of these eight patients were classified as having presumptive syphilis because no other etiology of their ulcers could be found, they were treated with benzathine penicillin alone, and resolution of their ulcers was noted following treatment. Three patients for whom darkfield microscopy or a DFA test was positive and serological tests were negative did not return for posttreatment evaluation; they were considered to have had presumptive syphilis.

Evidence for herpes simplex virus was found in the genital ulcers of 35 (43%) of the 82 patients (table 3). Definite herpes simplex (defined by a positive culture) was noted in 24 persons (69%); DFA tests for all of these patients were also positive. A presumptive diagnosis of herpes simplex virus infection was made for 11 patients (31%) for whom a DFA test was positive but culture was negative. Tzanck tests were specific but not sensitive. We did not recover *C. trachomatis* from any lesions.
nor did we encounter any lesions suggestive of donovanosis (granuloma inguinale).

An interesting finding was that conclusive evidence for more than one microbial etiology of the genital ulcer(s) was found in 19 (23%) of the 82 patients (table 4). A single etiology of the genital ulcer was certain in 46 patients (56%). No definitive diagnosis could be reached for 17 patients (21%), yet there was presumptive evidence of at least one infection (either alone or in combination with a definite infection) in 14 of these patients. No evidence of infection was found for only three of the 82 patients.

As detailed in table 4, the most common diagnosis was syphilis; T. pallidum was the sole etiology of the ulcer in 18 (22%) of the 82 patients, and herpes simplex virus was the sole etiology in 17 (21%) of the patients. The third most frequent diagnosis was the combination of primary syphilis and chancroid (12 patients [15%]). H. ducreyi was the sole pathogen in 11 patients (13%), and it was found in combination with herpes simplex virus in four women (5%). Finally, three women (4%) were found to have simultaneous primary syphilis and herpes simplex virus infection.

Discussion

This report describes a prospective evaluation of the etiology of genital ulcer disease in Brooklyn. The data reflect the epidemics of the 1980s: HIV disease and crack cocaine use. By utilizing contemporary diagnostic techniques, such as fluores-

cent monoclonal antibody staining and improved methods for culturing H. ducreyi, we were able to identify a definite microbiological etiology in 79% of cases.

The finding of multiple infections was striking, with 23% of patients having unequivocal evidence of two microbial etiologies. Dual infection with syphilis and chancroid was the third most common diagnosis. Previous African studies have documented that the rates of multiple infections are between 10% and 14% [6, 7]. However, these percentages may be falsely low since newer diagnostic tools, such as DFA staining, were not utilized.

Our finding of multiple etiologies of genital ulcers is interesting as incubation periods for the three infections vary from 2–7 days for herpetic simplex to as long as 90 days for primary syphilis. Therefore, either single infections were obtained from multiple sexual partners over time or multiple infections were obtained simultaneously, with delay in seeking treatment and subsequent presentation with concurrent diseases. Both explanations are plausible for our study population.

The average number of sexual partners in the preceding month and the preceding year for the 65 patients who volunteered the information was 1.9 (range, 0–40) and 10.6 (range, 1 to >300), respectively. Patients who had primary syphilis and chancroid reported an average of 4.1 sexual partners in the preceding month and 35.5 sexual partners during the preceding year. These numbers are probably falsely low, as many patients did not consider oral sex as a risk factor for sexually transmitted disease and did not report those sexual partners with whom genital intercourse did not take place. In addition, there was a delay between appearance of the lesion and seeking medical care. This interval ranged from an average of 8.4 days for those patients for whom herpetic simplex virus infection was diagnosed to 24.2 days for those patients for whom primary syphilis and chancroid were diagnosed.

This long interval may explain an unexpected finding in our study. It is notable that none of the patients with primary syphilis had positive darkfield microscopy (or a DFA test) and a positive FTA-ABS test but a negative RPR test at presentation. A previous study documented that 18% of patients with primary syphilis had a positive darkfield FTA-ABS test and a negative RPR test [22]. This phenomenon has been explained by the fact that the FTA-ABS test becomes positive earlier than the RPR test. In our study, the long delay between appearance of the ulcer and seeking medical care may have resulted in positive FTA-ABS and RPR tests for the 33 patients with primary syphilis.

Our rate of recovery of herpetic simplex virus in cultures of lesion specimens with use of immunofluorescence was 69%; a rate of 82% was found in an earlier study [16]. This difference probably reflects the fact that the specimens for viral cultures in our study were taken from ulcers rather than from unroofed vesicles. In spite of a study bias against herpetic simplex by

Table 4. Genital ulcer diseases in 82 men and women in Brooklyn.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Women (n = 47)</th>
<th>Men (n = 35)</th>
<th>Total (n = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definite single infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>11 (23)</td>
<td>7 (20)</td>
<td>18 (22)</td>
</tr>
<tr>
<td>Genital herpes</td>
<td>9 (19)</td>
<td>8 (23)</td>
<td>17 (21)</td>
</tr>
<tr>
<td>Chancroid</td>
<td>4 (9)</td>
<td>7 (20)</td>
<td>11 (13)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>24</td>
<td>24</td>
<td>48 (56)</td>
</tr>
<tr>
<td><strong>Definite mixed infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis and chancroid</td>
<td>7 (15)</td>
<td>5 (14)</td>
<td>12 (15)</td>
</tr>
<tr>
<td>Chancroid and genital herpes</td>
<td>4 (9)</td>
<td>0</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Primary syphilis and genital herpes</td>
<td>3 (6)</td>
<td>0</td>
<td>3 (4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>14</td>
<td>5</td>
<td>19 (23)</td>
</tr>
<tr>
<td><strong>No definite diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only presumptive infection</td>
<td>8 (17)</td>
<td>6 (17)</td>
<td>14 (17)</td>
</tr>
<tr>
<td>No microbial evidence</td>
<td>0</td>
<td>3 (9)</td>
<td>3 (4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8</td>
<td>9</td>
<td>17 (21)</td>
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attempting to exclude typical recurrent lesions, isolated herpes simplex was the second most common cause of ulceration after primary syphilis alone.

It is important to note that the Tzanck test for herpes simplex was predictive for only five (21%) of 24 patients for whom cultures were positive. These data support the insensitivity of the test. It is frequently used as the sole diagnostic test in public sexually transmitted disease clinics. Given these findings, culture for herpes simplex virus should be routinely offered in all clinics.

Our rate of recovery of *H. ducreyi* from lesions for which gram staining was positive was 68%; this rate is comparable with the rates of positivity of cultures of specimens from suspicious lesions that ranged from 61% to 81% in African studies [20]. The high incidence of chancroid is consistent with reports from New York City since 1985 [15]. Many of the patients with chancroid also had syphilis, and, in fact, if presumptive data are included, chancroid with syphilis was more common than either disease alone.

Of note was the preponderance of women in our study population, which was probably multifactorial. Our clinic receives referrals from the hospital emergency department and primary care clinics as well as from neighborhood shelters. In these settings, clinicians are probably more likely to evaluate and treat male patients than female patients because of the relative ease in examination of men. Another potential explanation for the preponderance of women in our study population may be the use of crack cocaine. Crack cocaine use has been associated with high risk behavior for sexually transmitted diseases. This association was evident in our study. Crack cocaine use was reported by 27 patients, of whom 21 were women (P < .01).

This study also confirmed previously published reports indicating an association between crack cocaine use and syphilis [23, 24]. Evidence of primary syphilis was found in 18 (67%) of 27 crack cocaine users. Serological tests for syphilis were positive for an additional five crack cocaine users. Therefore, 23 (85%) of 27 crack cocaine users had evidence of infection with *T. pallidum*.

Testing for antibody to HIV was voluntary; informed consent and counseling before testing were required. Counseling after testing was performed when patients returned for their results. Our rate of HIV infection (16%) corresponds to our previously reported HIV seropositivity rate of 18% among all patients attending our sexually transmitted disease clinic, yet this rate is much lower than our previously reported seropositivity rate among patients for whom genital ulcer disease was diagnosed (26%) [21].

Our lower rate reflects an HIV seroprevalence among patients with first episodes of genital ulcer disease, whereas the previously reported higher rate was found among all patients with genital ulcer disease (including those with recurrent disease). Unprotected heterosexual intercourse was the presumed mode of transmission in eight (73%) of 11 HIV-positive individuals. Of note, crack cocaine use was reported by six (75%) of eight HIV-positive individuals without traditional risk factors for HIV infection.

In summary, we noted a high incidence of multiple infections in patients with genital ulcers, particularly coinfection with syphilis and chancroid. Coinfection may have been associated with multiple sexual partners and crack cocaine use as well as with delay in seeking medical treatment. These findings underscore the need for both clinical suspicion of multiple infections in patients with genital ulcers and comprehensive testing for all suspicious etiologies. It is clear that for our population of patients, safer sexual practices need to be taught and reinforced. Given the previously noted association between genital ulcer disease and HIV disease, longitudinal studies are needed to ascertain the risk for HIV infection in patients with genital ulcers.

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References


