Comparison of Clinical Diagnosis and Standard Laboratory and Molecular Methods for the Diagnosis of Genital Ulcer Disease in Lesotho: Association with Human Immunodeficiency Virus Infection


A multiplex polymerase chain reaction (M-PCR) assay for Haemophilus ducreyi, Treponema pallidum, and herpes simplex virus (HSV) was compared with clinical and standard laboratory methods for the diagnosis of genital ulcer disease (GUD) in 105 patients; 36% were human immunodeficiency virus (HIV)-seropositive. Chancroid (80%), syphilis (8%), and genital herpes (8%) were the most frequent diagnoses. H. ducreyi and HSV were isolated from ulcers of 43% and 18% of patients, respectively; in 35%, all cultures were negative and the laboratory diagnosis indeterminate. M-PCR detected H. ducreyi, T. pallidum, and HSV in 56%, 23%, and 26% of patients, respectively; (no definitive diagnosis, 6%). The proportion of patients with more than one agent was 4% by culture and 17% by M-PCR (P = .002). Resolved sensitivities of M-PCR for H. ducreyi and HSV cultures were 95% and 93%, respectively. The sensitivities of H. ducreyi and HSV cultures were 75% and 60%, respectively. HSV, detected in 47% of specimens from HIV-infected versus 16% from HIV-uninfected patients (P < .001), may be emerging as a more frequent cause of GUD.

Sexually transmitted diseases (STDs) facilitate the transmission of human immunodeficiency virus (HIV) [1]. Although genital ulcer disease (GUD) has been recognized as a major risk factor in HIV transmission [2, 3], only recently has effective treatment of GUD and other STDs been shown to reduce the incidence of HIV infection in Africa [1]. However, atypical ulcers, HIV infection, and the presence of multiple agents can markedly affect the accuracy of a clinical diagnosis of GUD and the efficacy of treatment.

Ideally, appropriate treatment of GUD should be provided following determination of the etiology of the genital ulcer by laboratory investigations [4–6]. However, laboratory tests are often not done. These diagnostic limitations have been overcome by the introduction of a syndromic approach to GUD management worldwide [7]. Syndromic management takes into account the relative importance of the causative microorganisms of a disease syndrome and their antimicrobial susceptibilities, but it requires a knowledge of GUD etiology in the application of laboratory tests that are both sensitive and specific.

The etiology of GUD varies both geographically and temporally [8–10]. The three primary agents causing GUD in STD clinic patients are Treponema pallidum, Haemophilus ducreyi, and herpes simplex virus (HSV). Laboratory tests for the detection of these organisms are relatively insensitive and are often not available in clinics where GUD patients are seen [11].

The development of nucleic acid amplification technologies, such as the polymerase chain reaction (PCR), has resulted in sensitive and specific methods for the detection of many infectious agents. Separate PCR assays have been used to detect the presence of T. pallidum, H. ducreyi, and HSV [12–15]. The present study compared a multiplex PCR (M-PCR) amplification assay, which can simultaneously detect the presence of these three organisms in a single ulcer specimen [10], with results of conventional laboratory tests with a goal to assess the accuracy of a clinical diagnosis and study the association between GUD and HIV infection in an African STD clinic population with a high prevalence of HIV infection.

Materials and Methods

Patients and definitions for clinical diagnoses. Genital ulcer specimens were obtained from 105 consecutive patients with GUD presenting to the Genitourinary Medicine Clinic, Queen Elizabeth II Hospital, Maseru, Lesotho, between October 1993 and January 1994.

The criteria used for clinical diagnoses were defined prospectively. Lesions that were deep, purulent, and painful, with irregular raised red margins, were diagnosed as chancroid. Syphilis was...
diagnosed when ulcers were nontender, avascular, and indurated, with smooth nonpurulent bases. Genital herpes was diagnosed when ulcers were small and superficial, with or without vesicles or a history of recurrence (or both). Exuberant, velvety-red, non-painful lesions were diagnosed as donovanosis (granuloma inguinale). Lymphadenopathy was documented if inguinal and femoral lymph nodes were \( \geq 15 \) mm in diameter. When painful regional lymphadenopathy was found in association with a single, small primary lesion, a diagnosis of lymphogranuloma venereum (LGV) was made. Similarly, lymphadenopathy found in association with larger, painful, purulent lesions was defined as chancroid. Bilateral, discrete, nontender lymph nodes were regarded as exudative chancroid. Bilateral, discrete, nontender lymph nodes were regarded as exudative chancroid. Bilateral, discrete, nontender lymph nodes were regarded as exudative chancroid. Bilateral, discrete, nontender lymph nodes were regarded as exudative chancroid.

A definitive diagnosis by standard laboratory tests was based on the results of cultures for \( H. ducreyi \), HSV, and \( Chlamydia trachomatis \), so that all cultures had an equal chance of being from the first, second, or last swab \[16, 17\]. \( H. ducreyi \) was isolated by directly inoculating the specimen onto two solid selective media, as previously described \[17\]. Cell cultures for \( C. trachomatis \) and HSV were done using cycloheximide-treated McCoy cells and human embryo lung fibroblasts, respectively. Material was collected from the edges of the ulcer (or of the largest ulcer, if more than one was present) with a platinum scraper, spread on a microscope slide, fixed with methyl alcohol, stained (Giemsa), and examined for the presence of Donovan bodies. Darkfield microscopy of lesion specimens was not done; a direct fluorescent antibody test for \( H. ducreyi \) was attempted but was abandoned due to problems with slide fixation and storage.

An additional specimen was collected from the base of each lesion with a cotton-tipped swab for use in the PCR assays, which were performed at the Centers for Disease Control and Prevention and Roche Molecular Systems. Each swab was expressed into 0.2 mL of sterile distilled water, which was stored frozen at \(-70\)°C until analyzed.

Serum specimens obtained by venipuncture were tested for antichlamydial antibody by the microimmunofluorescence (MIF) technique. Titters ≥1:256 were considered diagnostic for LGV if the antibody detected was shown to be broadly cross-reactive \[18\]. In addition, serum was tested by quantitative rapid plasma reagin test (RPR; Omega Diagnostics, Alloa, UK) and fluorescent treponemal antibody absorption test (FTA-ABS; Murex, Dartford, UK).

HIV serology was done by ELISA (Abbott Laboratories, Abbott Park, IL); low-positive results were confirmed by Western blot (HSV blot 2.2; Diagnostic Biotechnology, Singapore) and high-positive results were confirmed by direct fluorescent antibody test (DFA; Serofluor; Virion, Rüschlikon, Switzerland).

A definitive diagnosis by standard laboratory tests was based on the results of cultures for \( H. ducreyi \), HSV, and \( C. trachomatis \) and MIF for LGV. If none were positive, the laboratory diagnosis was defined as indeterminate. Because of the absence of darkfield microscopic or DFA results, patients with negative or contaminated cultures, including those with reactive serologic tests for syphilis, were considered to have an indeterminate laboratory diagnosis.

HSV type-specific antibodies were determined as described using Western blot analysis of recombinant-derived, baculovirus-expressed HSV glycoproteins G1 and G2 from HSV-1 and -2-infected cells, respectively \[19, 20\].

Preparation of specimens for M-PCR. A 0.1-mL aliquot of each swab specimen was diluted with 0.4 mL of specimen transport medium (AmpliC; Roche Diagnostic Systems, Branchburg, NJ) containing 1 \( \mu \)g/mL calf thymus DNA (Sigma, St. Louis) and then heated at 95°C for 10 min. Subsequently, the specimen was extracted with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1), followed by chloroform–isoamyl alcohol (24:1). The nucleic acids were precipitated with ethanol, resuspended in 220 \( \mu \)L of a 1:1 mixture of specimen transport medium and specimen diluent (AmpliC) containing Tween 20 and magnesium chloride, and heated at 55°C for 10 min prior to PCR analysis.

A "mock" clinical specimen consisting of buffer was included in every set of nucleic acid extractions. A portion of the processed specimen was used in the \( C. trachomatis \) PCR test (AmpliC) \[21\].

PCR amplifications. M-PCR incorporating an internal control plasmid to monitor PCR inhibition was performed as described previously, with the exception that 10 U of Taq polymerase (AmpliTaq; Perkin-Elmer Cetus, Norwalk, CT) was used \[10\]. All amplifications were done in duplicate for each patient specimen. Appropriate precautions were taken to prevent contamination. Analysis of each set of 20 specimens included 2 negative controls lacking DNA, a negative control for specimen processing, and positive controls consisting of mixtures of DNA from 3 plasmids containing cloned fragments of the target genes at a concentration of 10–100 copies per reaction. Colorimetric detection and identification of amplification products were done as described \[10\]. Specimens with split positive-negative results were reanalyzed in duplicate, and a specimen was considered positive if >50% of the amplifications produced positive results. Specimens in which both duplicates gave an \( A_{260} \) reading >0.25 were considered positive.

HSV amplified products were typed using immobilized type-specific capture probes \[22\]. Classification of genital herpes as primary, nonprimary, or recurrent was based on HSV serology and DNA typing results \[23\].

Comparison of laboratory and clinical diagnoses. Sensitivity and specificity of M-PCR were calculated using culture for \( H. ducreyi \) and HSV as the reference standard. Sensitivity and specificity were calculated for the clinical diagnoses of chancroid and genital herpes using positive results of culture and M-PCR as reference standards and for that of syphilis using results of M-PCR and serology as reference standards.

Specimens for which M-PCR results were discrepant with the results obtained from HSV or \( H. ducreyi \) culture were reanalyzed as described \[10\]. Specimens were resolved as positive if they were culture-positive for \( H. ducreyi \) or HSV or if they were negative by culture but consistently positive for \( H. ducreyi \) or HSV by repeat M-PCR and confirmatory PCR assay.

Statistical analysis. Demographic and clinical data and results of laboratory tests were abstracted onto a standardized form and analyzed using Epi Info version 6 (CDC).
Results

Clinical evaluation. The patient population consisted of 69 men (66%) with a median age of 24 years (range, 16–50) and 36 women (34%) with a median age of 23 years (range, 17–40). Clinical diagnoses were recorded for 103 patients: chancreoid in 84 (81%), syphilis in 8 (8%), genital herpes in 8 (8%), LGV in 2 (2%), and Donovanosis in 1 (1%).

Laboratory evaluation. Of 104 patients whose serum was tested for antibody to HIV, 37 (36%) were positive. Cultures for H. ducreyi, HSV, or C. trachomatis were positive for 64 patients (table 1). H. ducreyi was isolated from 44 (42%) of 104 specimens, HSV from 18 (18%) of 103 specimens, and C. trachomatis from 4 (4%) of 103 specimens. Twenty-four men (35%) and 10 women (28%) had negative cultures for H. ducreyi, HSV, and C. trachomatis; however, 3 had MIF titers ≥1:256 and were diagnosed with LGV (table 1).

Of 67 patients with a definitive diagnosis by standard laboratory tests, 63 (94%) had a single etiologic agent and 4 (6%) had two etiologic agents identified. Chancreoid was diagnosed in 44 patients (66%), HSV in 18 (27%), and LGV in 9 (13%) with a definitive diagnosis.

Table 1. Diagnosis of GUD as determined by standard laboratory tests and polymerase chain reaction (PCR) in 105 patients in Lesotho.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Standard laboratory test results, n (%)</th>
<th>PCR results, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. (%)</td>
<td>no. (%)</td>
</tr>
<tr>
<td>Chancroid</td>
<td>41 (39)</td>
<td>41 (41)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0</td>
<td>16 (16)</td>
</tr>
<tr>
<td>GH</td>
<td>16 (16)</td>
<td>17 (17)</td>
</tr>
<tr>
<td>LGV</td>
<td>6 (6)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Chancroid + LGV</td>
<td>2 (2)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Chancroid + GH</td>
<td>1 (1)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Chancroid + syphilis</td>
<td>0</td>
<td>5 (5)</td>
</tr>
<tr>
<td>GH + syphilis</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Chancroid + syphilis + GH</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>GH + LGV</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Indeterminate†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>31 (30)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Contaminated</td>
<td>5 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Missing specimens</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

NOTE. GH, genital herpes; LGV, lymphogranuloma venereum.

* Culture was used to detect Chlamydia trachomatis, Haemophilus ducreyi, and herpes simplex virus (HSV); microimmunofluorescence titer ≥1:256 was considered diagnostic of LGV.

† PCR (Ampli; Roche Diagnostics Systems, Branchburg, NJ) was used to detect C. trachomatis for LGV diagnosis; multiplex PCR was used to detect Treponema pallidum, H. ducreyi, and HSV.

‡ Serology (rapid plasma reagin and fluorescent treponemal antibody absorption tests) did not definitely differentiate current infection related to GUD from past infection.

§ Because of absence of darkfield microscopic or direct fluorescent antibody results, patients with negative or contaminated cultures were considered to have indeterminate laboratory diagnosis.

In PCR assays, the proportion of patients with indeterminate laboratory diagnoses decreased from 35% to 6% of 100 patients from whom specimens were available; less than half of this decrease was due to the establishment of a definitive diagnosis of syphilis (table 1). The proportion of patients with multiple agents increased from 4 (4%) of 103 to 17 (17%) of 100 (P = .002). M-PCR detected T. pallidum either alone or in the presence of H. ducreyi or HSV in 23 (23%) of 100 patients. Either alone or in combination with another agent, H. ducreyi was detected in 56 and HSV in 26 patients. C. trachomatis was detected by PCR in 7 patients.

M-PCR identified 39 (93%) of 42 H. ducreyi culture-positive specimens; of the 3 M-PCR-negative isolates was available for further study. DNA from this strain was amplified by M-PCR. All 3 culture-positive M-PCR-negative specimens were also negative in the confirmatory PCR assay for H. ducreyi. There were 14 M-PCR-positive specimens that were negative by culture; all 14 gave a positive result in the confirmatory assay and were resolved as true positives. The unresolved specificity of M-PCR for H. ducreyi was 73%. The resolved sensitivity and specificity were 95% and 100%, respectively. Using the resolved data, the sensitivity of H. ducreyi culture relative to M-PCR was 75%.

M-PCR identified 15 of 17 HSV culture-positive specimens (an unresolved sensitivity of 88%) and was positive in an additional 10 culture-negative specimens. Both of the culture-positive M-PCR-negative specimens were negative in the confirmatory PCR assay. All 10 culture-negative M-PCR-positive specimens were positive in the confirmatory PCR assay and were resolved as true positives. The unresolved specificity was 88%. The resolved sensitivity and specificity of M-PCR were 93% and 100%, respectively. Using the resolved data, the sensitivity of HSV culture relative to M-PCR was 60%; 9 of 10 culture-negative M-PCR-positive specimens were from ulcers with multiple etiologic agents.

Among 67 patients with diagnoses established by standard laboratory tests, 11 (16%) had reactive serologic tests for syphilis, whereas among 36 with negative or contaminated cultures or negative MIF (indeterminate diagnosis), 14 (39%) had reactive serologic tests for syphilis (P = .01). Patients with indeterminate diagnoses were significantly more likely to have ulcers positive for T. pallidum by M-PCR assay than those with another definitive diagnosis; the strength of this association varied by RPR and FTA-ABS test results (table 2). There were no patients with an indeterminate diagnosis and nonreactive serologic test results who had positive M-PCR results for T. pallidum. FTA-ABS tests were reactive in 21 (91%) of 23 patients with positive M-PCR results for T. pallidum; in contrast, RPR tests were only reactive in 13 (57%) of these 23 patients (P = .007). T. pallidum M-PCR results were negative in 42 (67%) of 63 patients with reactive RPR or FTA-ABS test results.

Neither patient diagnosed clinically with LGV had C. trachomatis detected in ulcer specimens by culture or PCR or had
Table 2. Rates of detection of Treponema pallidum by multiplex polymerase chain reaction (M-PCR) among 105 patients with definite and indeterminate laboratory diagnoses: total and stratified by rapid plasma reagin (RPR) and fluorescent treponemal antibody absorption (FTA-ABS) test reactivity.

<table>
<thead>
<tr>
<th>Serologic test results, *</th>
<th>Positive T. pallidum M-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indeterminate laboratory diagnosis</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>RPR/FTA-ABS</td>
<td></td>
</tr>
<tr>
<td>-/+</td>
<td>13</td>
</tr>
<tr>
<td>+/+</td>
<td>13</td>
</tr>
<tr>
<td>-/-</td>
<td>9</td>
</tr>
<tr>
<td>+/-</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
</tr>
</tbody>
</table>

* Nonreactive (−) or reactive (+); excludes 5 patients without PCR or serologic (or both) test results.

† Prevalence ratio of positive M-PCR for T. pallidum in patients by laboratory diagnosis (definite vs. indeterminate), stratified by RPR and FTA-ABS results.

‡ Fisher’s exact 2-tailed; NS, not significant.

MIF titers ≥1:256. Among 3 patients with MIF titers ≥1:256, 1 had C. trachomatis and H. ducreyi detected by PCR. C. trachomatis was isolated by cell culture from ulcer specimens of 4 patients (table 1); none had MIF titers ≥1:256.

Sensitivity and specificity of clinical diagnoses. The sensitivity of a clinical diagnosis of chancroid among patients with a single etiology was >95% relative both to culture and to M-PCR, but the specificity of the diagnosis was 29% and 41%, respectively. Conversely, the sensitivities of clinical diagnoses of genital herpes and syphilis were 47% and 38% relative to HSV culture and to HSV and T. pallidum M-PCR, respectively, but the specificity of both was 98%; 1 of 8 patients with a diagnosis of genital herpes had H. ducreyi detected by M-PCR.

Effect of HIV infection on the diagnosis of GUD. HSV was detected in a significantly higher proportion of HIV-seropositive than HIV-seronegative patients (47% vs. 16%, P < .001), while T. pallidum was detected significantly less often in HIV-seropositive patients (9% vs. 32%, P = .011; table 3). In patients with only one agent detected by M-PCR, the proportion of accurate clinical diagnoses of genital herpes infection (as defined by M-PCR HSV results) was significantly lower among HIV-positive patients (71% vs. 92%, P = .029), but accuracy did not vary significantly for clinical diagnosis of chancroid or syphilis in HIV-positive or -negative patients.

Sero prevalence of HSV infection and relationship to HIV infection. Most patients (94/99 [95%]) had HSV-1 antibody, whereas only 55% (54/99) had HSV-2 antibody; only 3% (3/99) lacked antibodies to either (table 4). Men were significantly more likely than women to have antibodies to only HSV-1 (35/68 [52%] vs. 7/31 [23%], P = .007) and less likely to have antibodies to both (29/68 [43%] vs. 23/31 [74%], P = .004). Patients with antibody to HSV-2 (25/54 [46%]) were more likely to be HIV-seropositive than others (11/45 [24%]; prevalence ratio, 1.9; P = .024).

Most of the GUD patients with a laboratory diagnosis of genital herpes had antibodies to either HSV-1 (11/25 M-PCR-positive) or to both HSV-1 and -2 (13/25 M-PCR-positive); only 1 lacked antibodies to either. Specimens from 20 of these patients were available for HSV typing; 1 specimen had HSV-1 DNA and 19 had HSV-2 DNA. On the basis of a combination

Table 3. Rates and prevalence ratios (PRs) of positive multiplex polymerase chain reaction (M-PCR) for herpes simplex virus (HSV), Haemophilus ducreyi, and Treponema pallidum in 105 persons with GUD by HIV serostatus.

<table>
<thead>
<tr>
<th>HIV serostatus</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>PR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-PCR results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>16/34 (47)</td>
<td>10/63 (16)</td>
<td>3.0</td>
<td>.001</td>
</tr>
<tr>
<td>H. ducreyi</td>
<td>17/34 (50)</td>
<td>37/63 (59)</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>T. pallidum</td>
<td>3/34 (9)</td>
<td>20/63 (32)</td>
<td>0.3</td>
<td>.011</td>
</tr>
</tbody>
</table>

NOTE. Excludes patients whose HIV serostatus was unknown (n = 3) or whose specimen for M-PCR was unavailable (n = 5). NS, not significant.
of antibody and typing results, there was 1 primary, 6 nonprimary, and 11 recurrent HSV-2 infections and 1 recurrent HSV-1 infection. Recurrent HSV-2 infections were more common among HIV-seropositive patients (9/11 vs. 2/8; \( P = .02 \)).

Discussion

The major findings of this study are that an identifiable cause can be found for most genital ulcers if a sufficiently sensitive test is used and that HSV is a more common cause than suggested by previous GUD studies in Africa, particularly among HIV-infected patients.

PCR provided a more reliable and sensitive diagnosis of GUD than either clinical examination or use of standard laboratory tests, such as culture and serology. More importantly, the use of M-PCR reduced the number of patients with an indeterminate laboratory diagnosis from 35% to 6%, while the number with multiple agents in their ulcers increased from 4% to 18%. Previous studies on the etiology of GUD in developing and developed countries found that by using standard laboratory tests, the proportion of patients with ulcers of unknown etiology varied from 9% to 37% [24–28].

However, it is difficult to compare the results of previous studies [24, 25], which used darkfield microscopy to assist in the diagnosis of syphilis, since it was not used in our study. It is likely that some of the ulcers without a definitive laboratory diagnosis would have been darkfield-positive. This possibility is supported by the observation that M-PCR detected \( T. pallidum \) in ulcer specimens from 15 of 35 patients with indeterminate diagnoses by standard laboratory testing. The use of serologic test results for syphilis without other diagnostic tests and prior history is relatively insensitive and nonspecific [29] and would result in an overdiagnosis of primary syphilis due to the high prevalence of untreated or recently treated syphilis in this population. A similar finding was recently reported during a chancroid epidemic in Jackson, Mississippi [30]. Nevertheless, FTA-ABS results were more effective in predicting \( T. pallidum \) infection among patients with an indeterminate laboratory diagnosis than were RPR test results, probably because of the low sensitivity of the RPR test in primary syphilis.

Other studies from southern Africa have suggested that LGV and donovanosis are relatively common causes of GUD. Both were uncommon in our series. This may be due to geographic and temporal variations in the etiology of GUD. The positive PCR tests for \( C. trachomatis \) in ulcer specimens possibly reflected contamination from a urethral or cervical infection. This possibility cannot be addressed, as isolates were not serotyped and concomitant urethral and cervical isolates were not available. MIF titers did not suggest that true cases of LGV were missed, however. Moreover, LGV does not generally present with an ulcer, but with isolated inguinal lymphadenopathy, so studies like ours, which primarily address GUD, would not be expected to have a large proportion of patients with LGV.

The primary factors responsible for the decrease in indeterminate diagnoses appear to be the increased sensitivity of M-PCR and its ability to detect \( T. pallidum \) in ulcer specimens. Contamination of specimens or carryover of amplified DNA targets (amplicons) is a potential concern when using DNA amplification assays. However, specimen contamination was minimized by following relevant guidelines [31, 32].

Prior and current GUD have been shown to be associated with HIV infection (reviewed in [33]). This association does not necessarily reflect a causal relationship, as both GUD and HIV infection may reflect a common risk factor (i.e., sexual exposure). The presence of antibody to gG2 in 54% of patients with GUD indicates that infection with HSV-2 was common in this population. However, only 17% of the patients currently had an ulcer from which HSV was the only agent detected. This prevalence is similar to that reported in a South African study [24] but is considerably less than that reported for patients with GUD attending an STD clinic in Uganda [34]. As in other studies, we found that HSV-2 antibody was significantly associated with being female and with being HIV-seropositive [19, 23]. For genital herpes, clinical symptoms are a poor indicator of infection [35]; most HSV-2–infected persons have few or no symptoms. Inapparent or unrecognized lesions might allow HIV access to susceptible target cells. HSV-2 has been
implicated as a risk factor for HIV infection in some [36] but not other studies [34, 37].

Several studies [38–41] have demonstrated that a past history of syphilis is a risk factor for HIV infection. However, we did not find an association between a positive FTA-ABS test result or syphilis as a cause of GUD infection and HIV infection, for reasons that are unclear.

The strong association between detection of HSV and HIV infection is consistent with the hypothesis that genital herpes may behave as an opportunistic disease in HIV-infected persons [42]. HSV nucleic acid was detected more frequently in ulcers of HIV-infected patients by M-PCR, even when clinical appearance and conventional laboratory techniques did not suggest the presence of HSV. HSV was detected by M-PCR in 10 culture-negative specimens, of which 9 were from ulcers with multiple agents. M-PCR may have detected the presence of a low number of viruses resulting from recent reactivation or asymptomatic shedding, which was too low to be detected by culture. Alternatively, specimens obtained from chancroidal or syphilitic ulcers often contain blood or serous material. Although this is much less likely, the presence of neutralizing antibodies to HSV-1 or to both HSV-1 and -2, which all of these patients had, may preclude viral growth in tissue culture.

This study has several limitations. The absence of darkfield microscopic and DFA results for T. pallidum clearly exaggerated the performance of the M-PCR test, particularly in reducing the proportion of patients with indeterminate test results. The applicability of these results to other patient populations, particularly in developed countries and settings other than STD clinics, may be limited. The very small number of patients, particularly with diagnoses other than chancroid, greatly reduced the precision of prevalence, sensitivity, and specificity estimates. Further studies are needed using PCR technology to assess etiology of GUD in other settings.

Clinical diagnosis functioned adequately, since most patients would have been treated with penicillin and an agent effective for chancroid, according to the recommendations in place [7, 16]. Nevertheless, chancroid was overdiagnosed and syphilis and genital herpes clearly underdiagnosed. Algorithms tend to depend on typical ulcer morphology and on single-pathogen etiology [7]. Moreover, ulcers with the clinical appearance of genital herpes are not treated; the possibility of coinfection with bacterial pathogens is not considered, even in populations with a high prevalence of syphilis and chancroid. Cases of bacterial GUD can be misdiagnosed as genital herpes and not treated, as in our study. Clearly, there are economic barriers to more aggressive diagnostic and therapeutic strategies for GUD in Africa, but evaluations of diagnosis of GUD in HIV-infected persons represent a potential benefit that should not be ignored.

As syndromic treatment for bacterial GUD is more widely implemented in Africa, HSV may be emerging as a more important factor in GUD etiology and HIV transmission [34]. Because HSV-2 infection was common in HIV-infected patients in this study, recurrences may be more common and infectious periods longer as the level of immunosuppression increases [42, 43]. A high prevalence of HSV-2 may limit the effectiveness of mass treatment of bacterial STDs as a means of preventing HIV infection, since HSV-2 is not eradicated by antibiotics used to treat bacterial STDs. While acyclovir suppresses recurrent herpetic infections, cost may preclude widespread use. Thus, counseling to encourage condom usage and reduction in the number of sex partners will be essential in lowering the number of HIV infections attributable to GUD.

References