Possible reasons for an increase in the proportion of genital ulcers due to herpes simplex virus from a cohort of female bar workers in Tanzania

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METHODS
The study protocol was approved by the ethics committees of the Tanzanian National Institute for Medical Research, Dar es Salaam, Tanzania, and the London School of Hygiene & Tropical Medicine, London, UK.

In 2000, an open cohort of 600 female bar workers was established in Mbeya Region, Tanzania. Participants were recruited among women working in bars, restaurants and guesthouses at 14 communities along highways in Mbeya Region, and followed up at 3-month intervals for up to 27 months. An additional 153 women were recruited to replace women lost to follow-up during the study. At each 3-month visit, participants were offered STI screening and on-the-spot syndromic STI treatment, health education, HIV voluntary testing and counselling, and condoms. Information on behaviours was collected through structured interviews. Participants underwent genital examinations and were serologically screened for syphilis, HIV and HSV-2 infection. Treatment for STI did not include antiviral drugs for HSV. HIV antiretroviral therapy has been introduced to the region recently, but was not yet available to people infected with HIV at the time of this study. Further details of the bar worker cohort and the HIV/STI prevention and care services provided have been reported elsewhere.

Disruptions of the genital epithelium were diagnosed as ulcers and vesicular lesions as blisters. The term GUS (used synonymously with genital lesions) was used to denote genital ulcers and/or blisters. A multiplex polymerase chain reaction

Abbreviations: GUS, genital ulcer syndrome; HSV, herpes simplex virus; HSV-2, herpes simplex virus type 2; PCR, polymerase chain reaction; STI, sexually transmitted infection.
(M-PCR was used for the detection of T pallidum, H ducreyi and HSV from a single dry ulcer swab in the Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.  

The HIV serostatus was determined through a dual-testing strategy (HIV-Determine, Abbott, Abbott Laboratories, Abbott Park, Illinois, USA, and Enzygnost HIV1+2 plus, Behring, Germany), and discrepant results were resolved by western blot (HIV Blot 2.2, Genelabs Diagnostics, Singapore, Singapore). HSV-2 IgG were detected with an enzyme immune assay (KALON Biological, Aldershot, UK).

Data from all 10 examination rounds were combined to assess the association between HIV status and GUS. A random effects logistic regression model was used to take account of repeated measurements on individual participants, and the model adjusted for other potential confounding factors including HSV-2 status. Odds ratios (ORs) for trend were used to evaluate trends in the prevalence of GUS across the 10 rounds of follow-up, but the proportional distribution of GUS aetiologies was assessed by grouping rounds 1–3, 4–6 and 7–10. The effect of different sexual behaviours on these trends was assessed by comparing crude ORs with adjusted ORs in turn for each of these factors. A subanalysis was performed on those women who seroconverted for HIV, to compare the odds of GUS occurring in the round before, at or after the first HIV-positive result, controlling for the study round.

RESULTS
Prevalence of HIV, HSV-2 and GUS
The prevalence of HIV, HSV-2 and HIV/HSV-2 coinfection among all 753 women at recruitment was 66.9%, 88.8% and 63.7%, respectively. At a total of 5762 examinations, GUS was diagnosed 312 times in 227 individual women (table 1). A single episode of GUS was recorded in 161 women, whereas 2–5 episodes were recorded in a further 66 women. Genital herpes was diagnosed at 116 examinations (2%), syphilitic lesions at 12 (0.2%) and chancroid at 7 (0.1%). No aetiological agent was found in 178 GUS cases (3.1%; table 1).

GUS aetiologies
A large proportion of GUS (178/312; 57%), including 31% of blisters and 64% of ulcers, was not attributable to detectable pathogens. HSV alone was detected in 115 (37%) lesions, T pallidum alone in 11 (4%) lesions and H ducreyi in 7 (3%) lesions. In addition, there was one mixed infection with HSV and T pallidum (0.3%). All the 17 lesions consisting of both ulcers and blisters were caused by HSV. Only 132 of 312 (42%) women were aware of their lesions. Awareness was greatest among women with genital herpes (51%) and lower in women with primary syphilis (5/11), chancroid (3/7) and lesions without aetiology (37%); however, these differences were not significant (p = 0.16).

Although 26% of all lesions and 50% of lesions without detectable aetiology were believed by clinicians to be of non-infectious (traumatic through scratching or shaving) origin, on the basis of the physical appearance, this was the case for none of the syphilitic and chancroidal lesions and for only 5% of herpetic lesions; 91% (41/45) of these lesions were pathogen negative.

Association of follow-up status and GUS
During the observation period, 153 women were lost to follow-up; 55 of these women died, of whom 54 were infected with HIV. The observed prevalence of GUS was highest among women who later died (13.9%), followed by women who dropped out for other or unknown reasons (8.8%) and lowest among women who completed follow-up (4.7%). These differences were statistically significant (p = 0.001 and p = 0.03, respectively). Women who died later were more likely to have herpetic lesions than women who completed follow-up (adjusted OR 4.22, 95% confidence interval (CI) 2.25 to 7.92).

This association remained when the analysis was restricted to HIV-positive women (adjusted OR 3.05, 95% CI 1.62 to 5.71).

Association of HIV status and GUS
Excluding the round of HIV seroconversion to exclude GUS due to acute seroconversion illness, GUS was diagnosed at 277 (6.9%) of 4007 examinations of HIV-positive women, compared with 32 (1.9%) of 1714 examinations of HIV-negative women (OR 4.02, 95% CI 2.71 to 5.95; table 2). Except for syphilitic ulcers, both pathogen-positive and pathogen-negative lesions were more prevalent among HIV-positive women with crude ORs in the range 3.5–6.2. Chancroid was seen only in HIV-infected women. Including the round of HIV seroconversion had a modest effect on the strength of the association between GUS and HIV infection (table 2).

The association between HIV and GUS was stronger in HSV-2 seropositive women (OR 4.02, 95% CI 2.61 to 6.19) than in HSV-2 seronegative women (OR 2.55, 95% CI 0.68 to 10.97); however, this difference was not significant (test for interaction: p = 0.44). The association between HIV and GUS was still highly significant after adjusting for HSV-2 status (table 2), and was not due to confounding by differences in reported sexual behaviour between HIV-positive and HIV-negative women.

Frequency of GUS in relation to HIV seroconversion
The prevalence of GUS at the approximate time of HIV seroconversion (8.3%; 3/36 examinations) was higher than at visits after seroconversion (5.3%; 11/208 examinations) or before seroconversion (2.0%; 2/99 examinations). Compared with the risk of GUS before HIV seroconversion and after adjusting for the examination round, ORs for GUS risk were 4.94 (95% CI 0.78 to 31.22; p = 0.09) at the approximate time of seroconversion and 5.72 (95% CI 1.05 to 31.11; p = 0.06) at visits after seroconversion. However, the number of HIV seroconversions was small, and overall these differences were not significant (p = 0.1).

Prevalence trends of GUS over 10 examination rounds
The prevalence of GUS decreased from 10.2% at the first examination round to 1.2% at the tenth round (ORtrend 0.85, 95% CI 0.82 to 0.89; table 3). Pathogen-negative GUS decreased markedly (ORtrend 0.78, 95% CI 0.73 to 0.83), partly due to a decrease in ulcers of presumed traumatic origin (ORtrend 0.69, 95% CI 0.58 to 0.82). The prevalence of genital herpes fluctuated around 2% (ORtrend 1, 95% CI 0.93 to 1.07), whereas
was observed in GUS in women later lost to follow-up (OR trend in women who completed follow-up). A non-significant increase with a relatively high prevalence of ulcers, trends in the potential loss to follow-up of women lost to follow-up (ORtrend 1.36, 95% CI 1.00 to 1.85), whereas the minimal effect on the rate of decline (ORtrend 0.84, 95% CI 0.81 to 1.07, 95% CI 0.95 to 1.2), and their exclusion had only a negligible effect on the rate of decline (ORtrend 0.99, 95% CI 0.93 to 1.06).

over time as immune deficiency progressed; no increase was observed (ORtrend 0.99, 95% CI 0.93 to 1.06).

To assess the possibility that the observed decrease in GUS could have been due to the selective loss to follow-up of women with a relatively high prevalence of ulcers, trends in the prevalence of GUS and genital herpes were analysed separately in HIV-negative women, because herpetic lesions could have increased over time as immune deficiency progressed; no increase was observed (ORtrend 0.99, 95% CI 0.93 to 1.06).

Changes in aetiological patterns of ulcerative STI over time

Table 4 shows changes in the proportional distribution of ulcer aetiologies over time. The proportion of GUS caused by HSV increased from 22.5% at examination rounds 1–3 to 58.4% at rounds 7–10 (ORtrend 2.58, 95% CI 1.71 to 3.89), whereas that of lesions without detectable aetiology decreased from 69.6% to 39% (ORtrend 0.46, 95% CI 0.32 to 0.68). These changes were independent of HIV status (tests for interaction of HIV status with trends in the proportional distribution of all three pathogens and pathogen-negative lesions; p=0.30. The data were reanalysed excluding pathogen-negative lesions. HSV was predominant from the start, causing an average of 73.8% pathogen-positive lesions during rounds 1–3 and increasing to 95.7% during rounds 7–10. The proportion of GUS caused by T pallidum and H ducreyi dropped from 14.3% and 11.9%,
Table 4  Changes in the proportional distribution of genital ulcer syndrome aetiologies over time

<table>
<thead>
<tr>
<th>STI</th>
<th>Period* 1 (rounds 1–3) n (%)</th>
<th>Period* 2 (rounds 4–6) n (%)</th>
<th>Period* 3 (rounds 7–10) n (%)</th>
<th>OR trend† (95% CI) p Value Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>All GUS (total n)</td>
<td>138</td>
<td>97†</td>
<td>77</td>
<td>2.58 (1.71 to 3.89) &lt;0.001 †</td>
</tr>
<tr>
<td>HSV (PCR)</td>
<td>31 (22.5)</td>
<td>40 (41.2)</td>
<td>45 (58.4)</td>
<td>to 3.89</td>
</tr>
<tr>
<td>T pallidum (PCR)</td>
<td>6 (4.4)</td>
<td>4 (4.1)</td>
<td>2 (2.6)</td>
<td>0.9 (0.11 to 7.65) 0.93 ↔</td>
</tr>
<tr>
<td>H ducreyi (PCR)</td>
<td>5 (3.6)</td>
<td>2 (2.1)</td>
<td>0 (0)</td>
<td>0.34 (0.09 to 1.3) 0.12 ↓</td>
</tr>
<tr>
<td>Pathogen-negative</td>
<td>96 (69.6)</td>
<td>52 (53.6)</td>
<td>30 (39)</td>
<td>0.46 (0.32 to 0.68) &lt;0.001 †</td>
</tr>
<tr>
<td>All pathogen-positive GUS (total n)</td>
<td>42</td>
<td>45†</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>HSV (PCR)</td>
<td>31 (73.8)</td>
<td>40 (88.9)</td>
<td>45 (95.7)</td>
<td>7.27 (4.0 to 131.15) 0.18 ↑</td>
</tr>
<tr>
<td>T pallidum (PCR)</td>
<td>6 (14.3)</td>
<td>4 (8.9)</td>
<td>2 (4.3)</td>
<td>0.18 (0 to 6.23) 0.34 ↓</td>
</tr>
<tr>
<td>H ducreyi (PCR)</td>
<td>5 (11.9)</td>
<td>2 (4.4)</td>
<td>0 (0)</td>
<td>0.22 (0.05 to 0.87) 0.03 ↓</td>
</tr>
</tbody>
</table>

GUS, genital ulcer syndrome; HSV, Herpes simplex virus; PCR, polymerase chain reaction; STI, sexually transmitted infection.

*One round is equivalent to a time period of 3 months, during which all participants were examined once.
†OR for linear trend with one unit increase in period using random effects logistic regression to allow for correlations between repeated measurements on individual women—see table 3 footnotes.
‡Including one lesion with mixed aetiology (HSV and T pallidum).

respectively, during the first period to 4.3% and 0% during the last period.

**DISCUSSION**

**GUS aetiologies**

The seroprevalences of HSV-2 (89%), HIV (67%) and HSV-2/ HIV coinfection (64%) were high among cohort participants. It is therefore not surprising that HSV was a frequent aetiology of GUS. As molecular typing of HSV was not done, it is possible that some herpetic lesions were caused by HSV type 1. However, a recent study using molecular typing on ulcer specimens from GUS patients in Dar es Salaam, Tanzania, did not find HSV type 1 in a single case. T pallidum was detected in only 12 (4%) and H ducreyi in 7 (2%) specimens collected from women with GUS. These proportions are low compared with findings of other studies carried out among patients with GUS in sub-Saharan Africa, but confirm results of a recent study among patients with GUS in Mbeya, where T pallidum was not found in any of 34 female patients and H ducreyi was only found in one single case (3%). It is possible that owing to a long standing STI intervention programme in Mbeya Region (in place since 1990), bacterial ulcerative STIs have already been effectively reduced, and this is supported by the steep decline in syphilis observed during the 1990s in antenatal care attendees.

The proportion of M-PCR negative results (57%) was unexpectedly high and decreased over time. In other studies using M-PCR, the proportion of pathogen-negative specimens ranges from 22% to 51%, and tends to be larger in population-based studies where genital ulcers are detected through screening of “healthy” individuals than in clinic-based studies of patients self-presentation with genital ulcers. Specimen taking was standardised as much as possible—for example, through clear case definitions of GUS and standardised sample collection procedures. However, if in doubt, study clinicians were likely to diagnose GUS and take a swab rather than risk missing a true STI case. Especially during the initial phase of the study, when the clinical team was inexperienced, this has certainly resulted to some extent in misclassification of other skin lesions as GUS. Furthermore, study clinicians suggested, on the basis of the physical appearance of ulcers, that a large proportion (26%) of lesions may have been provoked by women themselves through small traumata caused by shaving or scratching. As 91% (41/45) of these lesions were pathogen negative, this inference was often correct. Moreover, in some cases, pathogens may have been eradicated through antibiotic treatment before specimen taking and, lastly, the presence of additional aetiological agents, which cannot be detected by M-PCR, must be considered.

**Effect of HIV infection on the prevalence of GUS**

GUS and genital herpes specifically, were more prevalent among HIV-infected women. The association between genital herpes and positive HIV serostatus was strong as observed in many other cross-sectional and prospective studies. However, reports on pathogen-negative ulcers associated with HIV infection are rare, and we have no plausible explanation for this observation.

The prevalence of HSV-2 was higher among HIV-positive than among HIV-negative women, which could have been a simple explanation for the higher frequency of GUS among HIV-infected women. However, the higher prevalence of GUS in HIV-infected women was to a large extent independent of the HSV-2 status. This is consistent with HIV/HSV-2 coinfected women being at higher risk of developing herpetic lesions. Symptomatic herpes occurred especially frequently among women who died later probably of HIV-related causes, suggesting that advanced immune deficiency was associated with increased frequency of herpetic episodes. GUS with no detectable pathogen also occurred more frequently among women compared with women lost to follow-up for other or unknown reasons. This suggests that HIV and/or advanced immune deficiency may have caused or exacerbated lesions of unknown origin.

GUS occurred more frequently around the time of HIV seroconversion. Data do not allow us to determine whether genital lesions were present during the weeks before, at the same time as or during the weeks immediately after women acquired HIV. Nonetheless, the observations in Mbeya are consistent with the hypotheses that genital lesions may
increase susceptibility to HIV, that genital ulcers are a sign of acute HIV seroconversion illness and/or that HIV is frequently acquired from dually infected male partners (with HIV and ulcerative STI).

Decline in GUS over time
The prevalence of GUS among cohort participants declined gradually with successive examination rounds, and this was mainly due to a decrease in pathogen-negative lesions. The prevalence of syphilis and chancroid also declined, but case numbers were too small to contribute substantially to the overall decline in GUS. The decline in bacterial infections was probably real, as it coincided with declines in other treatable STIs such as gonorrhoea, chlamydia and trichomoniasis. The reduction in these STIs may be attributable to regular STI screening and treatment and education on HIV/STI prevention provided to cohort participants. A true reduction in lesions of non-infectious origin also seems possible, as women may increasingly have taken precautions to avoid traumata through scratching or shaving following advice from clinicians and nurses. In addition, however, the specificity of the diagnosis of GUS may have increased over time as clinicians became more experienced, resulting in declining proportions of participants diagnosed with GUS. Conversely, it cannot be excluded that some true cases of ulcerative STI were missed during later study rounds. It is reassuring that clinicians correctly attributed all syphilis and chancroid cases and the great majority of herps to an infectious origin as opposed to a mechanical origin, on the basis of clinical signs.

The risk of GUS, including that of genital herpes, chancroid and pathogen-negative lesions, was 2–5 times higher in women lost to follow-up than in those who remained in the study. The selective loss to follow-up of women at high risk of GUS may have contributed to the decline in prevalence of ulcers. If this were the case, the decline in GUS should have been less pronounced or non-existent if women lost to follow-up were excluded from data analysis. Such a difference was not observed with regard to GUS. However, the loss of women with frequent herpes episodes may have masked a genuine increase in the prevalence of genital herpes in the cohort over time.

Changes in the proportional distribution of ulcer aetiologies over time
The number of syphilis, chancroid and pathogen-negative cases decreased over time, whereas the number of genital herpes cases fluctuated around an average of 12 cases per examination round. This resulted in an increase in the proportion of GUS caused by HSV (although absolute numbers of genital herpes cases did not increase), and a proportional decrease in those caused by T. pallidum, H. ducreyi and of pathogen-negative cases. This is likely to reflect a scenario where bacterial STI are successfully controlled, although genital herpes remains uncontrolled and therefore becomes more predominant. The fact that the proportional increase in HSV as an aetiological agent occurred in HIV-infected and in HIV-uninfected women suggests that a potential increase in the incidence of herpetic episodes associated with progression of immune deficiency in HIV-infected women played only a limited role in these changes.

CONCLUSIONS
The changes observed in the frequency and proportional distribution of GUS aetiologies suggest that regular STI screening and treatment over an extended period can effectively reduce bacterial STI and should therefore be sustained. However, in populations with a high prevalence of HSV-2, there remains a considerable burden of genital herpes, which soon becomes the predominant cause of GUS. Given the observed associations between genital herpes and HIV transmission, high priority should be given to the evaluation of potential interventions to control HSV-2 either through a vaccine or through episodic or suppressive antiviral therapy.

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