ORIGINAL CONTRIBUTION

Genital Shedding of Herpes Simplex Virus Among Symptomatic and Asymptomatic Persons With HSV-2 Infection

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Context Since herpes simplex virus type 2 (HSV-2) antibody tests have become commercially available, an increasing number of persons have learned that they have genital herpes through serologic testing. The course of natural history of HSV-2 in asymptomatic, seropositive persons is uncertain.

Objective To evaluate the virologic and clinical course of HSV genital shedding among individuals with symptomatic and asymptomatic HSV-2 infection.

Design, Setting, and Participants Cohort of 498 immunocompetent HSV-2–seropositive persons enrolled in prospective studies of genital HSV shedding at the University of Washington Virology Research Clinic, Seattle, and Westover Heights Clinic, Portland, Oregon, between March 1992 and April 2008. Each participant obtained daily self-collected swabs of genital secretions for at least 30 days.

Main Outcome Measure The rate of viral shedding measured by quantitative real-time fluorescence polymerase chain reaction for HSV DNA from genital swabs.

Results Herpes simplex virus type 2 was detected on 4753 of 23 683 days (20.1%; 95% confidence interval [CI], 18.3%-22.0%) in 410 persons with symptomatic genital HSV-2 infection compared with 519 of 5070 days (10.2%; 95% CI, 7.7%-13.6%) in 88 persons with asymptomatic infection ($P<.001$). Subclinical shedding rates were higher in persons with symptomatic infection compared with asymptomatic infection (2708 of 20 735 days [13.1%; 95% CI, 11.5%-14.6%) vs 434 of 4929 days [8.8%; 95% CI, 6.3%-11.5%]) ($P<.001$). However, the amount of HSV detected during subclinical shedding episodes was similar (median, 4.3 [interquartile range, 3.1-5.6] $\log_{10}$ copies in the symptomatic infection group vs 4.2 [interquartile range, 2.9-5.5] in the asymptomatic infection group, $P=.27$). Days with lesions accounted for 2045 of 4753 days (43.0%; 95% CI, 39.8%-46.5%) with genital viral shedding among persons with symptomatic genital HSV-2 infection compared with 85 of 519 days (16.4%; 95% CI, 11.2%-23.9%) among persons with asymptomatic infection ($P<.001$).

Conclusion Persons with asymptomatic HSV-2 infection shed virus in the genital tract less frequently than persons with symptomatic infection, but much of the difference is attributable to less frequent genital lesions because lesions are accompanied by frequent viral shedding.

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HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) is one of the most frequent sexually transmitted infections worldwide, with global estimates of 536 million infected persons and an annual incidence of 23.6 million cases among persons aged 15 to 49 years.[1-3] In the United States, 16% of adults are HSV-2 seropositive,[4] but only 10% to 25% of persons with HSV-2 infection have recognized genital herpes.[5] Moreover, most HSV-2 infections are acquired from persons without a clinical history of genital herpes.[6] Thus, the risk of sexual transmission does not correlate with the recognition of clinical signs and symptoms of HSV-2 but most likely correlates with viral mucosal shedding.[7]

Previous work that characterized genital shedding in seropositive asymptomatic persons suggested that most, if not all, persons with HSV-2 intermittently shed virus on the genital skin or mucosa, and most such persons recognized recurrences after learning the clinical signs and symptoms caused by HSV-2.[5,8] However, the genital shedding rate was lower and the duration of lesional episodes was shorter among persons who were unaware of their HSV-2 status compared with those who had received a diagnosis of genital herpes. Whether these differences constitute perception, anatomic site, quantity of latently infected ganglionic cells, viral strain, or host immunity characteristics remains unclear. To better understand the biological differences be-

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tween persons who do and do not recognize their HSV-2 infection, we compared the rates and patterns of genital HSV shedding in what is to date, to our knowledge, the largest cohort of HSV-2–seropositive persons with and without a history of genital herpes, using daily sampling and an extensively characterized HSV polymerase chain reaction assay.

METHODS

Participants and Clinical Procedures

Participants were enrolled into prospective studies of natural history of genital HSV infection at the University of Washington Virology Research Clinic in Seattle and the Westover Heights Clinic in Portland, Oregon, between March 1992 and April 2008. Inclusion criteria were age 18 years or older, HSV-2 infection as defined by the University of Washington Western blot, and good general health. None of the participants received antiviral medication during the study period, and all participants obtained 30 or more days' worth of daily swabs of the genital skin/mucosa.

Participants were recruited from the community by word of mouth and advertisements. They were provided per-visit compensation for time and travel costs.

Asymptomatic participants were identified as potential participants in a study of a candidate prophylactic HSV-2 vaccine, but were unexpectedly found to be HSV-2 seropositive, through screening for HSV antibodies by their providers as part of medical care or had partners with genital HSV-2 but were thought to be uninfected. All participants signed informed consent and the University of Washington institutional review board approved the study protocol.

Demographic information and medical and sexual history were collected with standardized forms. Race and ethnicity information was gathered from participants by self-report. Participants attended individual educational sessions on HSV-2, were shown pictures of both typical and atypical lesions, and were instructed to inspect the genital region for lesions daily and obtain swabs of their genital area, including any genital lesions. Men were instructed to swab first the penile skin and then the perineum and the perianal areas.[9] Women were instructed to insert the swab into the vagina and then swab the vulva, the perineum, and the perianal areas.[10] Swabs were placed into vials containing 1 mL of polymerase chain reaction transport medium and stored at 4°C until laboratory processing. Each participant kept a diary of genital lesions and symptoms.[10] Participants visited the clinic every 2 weeks for symptom review and collection of swabs.

Laboratory Methods

Herpes simplex virus serostatus was determined with the University of Washington Western blot.[11] A real-time fluorescence-based quantitative polymerase chain reaction assay with primers to glycoprotein B was used to detect HSV DNA.[12] A result was considered positive if greater than 150 copies of HSV DNA per milliliter of polymerase chain reaction buffer were detected.[13] To detect contamination, 15 HSV-negative controls were included in each 96-well plate; no contaminants were detected.

Definitions

Participants were classified as having symptomatic HSV-2 infection if they had a clinical history of genital herpes at their diagnosis.[14] Participants had asymptomatic HSV-2 infection if they did not have a history of symptomatic genital herpes before the serologic diagnosis of HSV-2, and thus the diagnosis was based on the positive antibody test result for HSV-2. For most participants, the time of the serologic diagnosis coincided with study entry, but some participants may have received an HSV-2–seropositive test result before study entry. We further subdivided the participants without genital herpes but who had received a serologic HSV-2 diagnosis into those who remained asymptomatic and those who developed manifestations of genital herpes during the follow-up. Genital shedding was defined as detection of HSV in the genital area. Clinical viral shedding was defined as shedding on days with lesions consistent with genital herpes. Subclinical genital shedding was defined as days on which HSV was detected in the absence of lesions. We defined shedding episodes as consecutive HSV-positive swab results; those that were preceded and followed by 2 consecutive negative swab results were deemed to have known duration.[15]

Statistical Analysis

Genital shedding rates were calculated by using the number of days with positive swab results divided by the total number of days with swabs collected for each subject. Confidence intervals (CIs) for average shedding and lesion rates were computed with an intercept-only Poisson regression model, which correctly accounts for multiple samples per person. In cases in which raw rates and model-predicted rates differ by more than 1%, both are provided. The amount of virus shed per sample was quantified as $\log_{10}$ copies per milliliter. The annualized episode rate was calculated by scaling the number of shedding episodes to the follow-up period. Categories for variables such as age and recurrences per year were determined by using quantiles because graphic investigation demonstrated a lack of linear relationship. Years since acquisition were categorized according to previous work showing that shedding decreases the first year after HSV-2 acquisition and then stabilizes.[16] The Wilcoxon rank sum was used when continuous data were not normally distributed to calculate *P* values. The χ^2 test was used for categorical data, and the *t* test was used for normally distributed continuous data. To characterize the predictors of genital shedding, Poisson regression analyses were performed, including a scale parameter for overdispersion.[17] Variables that were hypothesized to be predictors of genital shedding included age, sex, race, sexual preference, HSV-1 seropositivity, frequency of herpes recur-

rences, and a history of symptomatic genital HSV-2 infection.[15,18] The multivariate model was determined through backward elimination. Variables with $P < .10$ in the univariate model were included in the initial multivariate model and then removed individually if $P > .05$. A 2-sided P value of .05 was considered to be statistically significant. A post hoc 2-sample test for repeated-measures binary data was performed to determine power: with outcome rates of 10% to 20%, we had at least 80% power to detect risk ratios equal to or less than 0.7 and equal to or greater than 1.2.[17] Statistical analyses were performed with SAS, version 9.2 (SAS Institute, Cary, North Carolina).

RESULTS

Characteristics of Study Participants

Four hundred ninety-eight HSV-2–seropositive, healthy participants collected at least 30 days' worth of genital swabs and were included in the analysis. The mean age of study participants was 40.7 years (SD, 11.0) (TABLE 1). Two hundred fifteen (43.2%) participants were men and 283 (56.8%) participants were women. Two hundred sixty-eight (53.8%) participants were HSV-2 seropositive only, whereas 230 (46.2%) participants were HSV-1 and HSV-2 coinfected. Most participants were white (401; 80.5%), 32 (6.4%) were African American, and 65 (13.1%) were other races or unknown. Most participants (325; 65.3%) were heterosexual and 68 (13.7%) were men who had sex with men; sexual behavior was not collected for 104 (20.9%). The mean age at sexual debut was 16.6 years (SD, 2.9).

Four hundred ten participants (82.3%) reported symptomatic genital herpes infection, whereas 88 (17.7%) participants were asymptomatic when they had serologically documented HSV-2 infection. Among persons with symptomatic genital herpes, the median time since HSV-2 infection was 8.3 years (interquartile range [IQR], 2.5-16.8) and the median number of self-reported recurrences per year was 4 (IQR, 2-8).

Table 1. Demographics, Clinical Characteristics, Frequency and Quantity of Genital Viral Shedding Rates, and Characteristics of Shedding Episodes (N = 498)

Characteristics	Value[a]	Asymptomatic HSV-2 Infection (n = 88)[b]	Symptomatic HSV-2 Infection (n = 410)[c]	*P* Value
Age, y, mean (SD)[d]	40.7 (11.0)	42.5 (9.6)	40.2 (11.3)	.06
Sex, No. (%)				
Men	215 (43.2)	54 (61.4)	161 (39.3)	<.001
Women	283 (56.8)	34 (38.6)	249 (60.7)	
Race, No. (%)				
White	401 (80.5)	73 (82.9)	328 (80.0)	.06
African American	32 (6.4)	9 (10.2)	23 (5.6)	
Other or unknown	65 (13.1)	6 (6.8)	59 (14.4)	
Sexual orientation, No. (%)				
Heterosexual men and women	325 (65.3)	48 (54.6)	277 (67.6)	<.001
Men who have sex with men	68 (13.7)	36 (40.9)	32 (7.8)	
Women who have sex with women	1 (0.2)	0	1 (0.2)	
Unknown	104 (20.9)	4 (4.6)	100 (24.4)	
Age at first sex, y, mean (SD)	16.6 (2.9)	16.0 (3.1)	16.7 (2.9)	<.001
HSV serology, No. (%)				
HSV-2 only	268 (53.8)	38 (43.2)	230 (56.1)	.03
HSV-1 and -2	230 (46.2)	50 (56.8)	180 (43.9)	
Years since initial infection, median (IQR)[e]	8.3 (2.5-16.8)		8.3 (2.5-16.8)	
Recurrences in year before first visit, median (IQR)	4.0 (2-8)	0 (0-4)[f]	4.0 (2-8)	<.001
Days sampled, median (IQR)	57.0 (47-62)	56.5 (43-67.5)	57.0 (50-62)	.83
PCR-positive days/total PCR swabs, No. (%)	5272/28 753 (18.3)	519/5070 (10.2)	4753/23 683 (20.1)	<.001
Subclinical PCR-positive days/subclinical swabs, No. (%)	3142/25 664 (12.2)	434/4929 (8.8)	2708/20 735 (13.1)	<.001
PCR-positive lesional swabs/total swabs with lesions present, No. (%)	2130/3089 (68.9)	85/141 (60.3)	2045/2948 (69.4)	.03
Genital $\log_{10}$ copies, median (IQR)[g]	4.9 (3.4-6.3)	4.5 (3.2-5.8)	4.9 (3.5-6.3)	<.001
Subclinical genital $\log_{10}$ copies, median (IQR)[g]	4.3 (3.1-5.6)	4.2 (2.9-5.5)	4.3 (3.1-5.6)	.27
Clinical genital $\log_{10}$ copies, median (IQR)[g]	5.6 (4.3-6.7)	5.7 (4.2-6.9)	5.6 (4.3-6.7)	.21
Genital shedding episode duration, median (IQR), d	3.0 (1.0-5.5)	2.0 (1.0-5.0)	3.0 (1.0-5.5)	.33

Abbreviations: HSV-2, herpes simplex virus type 2; IQR, interquartile range; PCR, polymerase chain reaction.
[a] Numbers may not sum to total because of missing data, and percentages may not sum to 100% because of rounding.
[b] Asymptomatic HSV-2 infection is defined as diagnosis of HSV-2 infection made by serologic testing in the absence of a history of genital lesions.
[c] Symptomatic HSV-2 infection is defined as clinical history of genital herpes at diagnosis.
[d] Age was unknown for 41 persons.
[e] For persons with history of genital herpes. Unknown for 48 persons with a history of genital herpes.
[f] One person who was asymptomatic at diagnosis by a positive HSV-2 antibody test result developed recurrences before study entry.
[g] Among positive samples.

Genital Shedding and Lesion Rate

Overall, there were 33 113 days of observation; of these, 28 753 had genital swabs available for analysis. HSV DNA was detected in 5272 of 28 753 swabs (18.3%; 95% CI, 16.7%-20.1%). Participants were observed for a median of 57 days (IQR, 47-62) (Table 1). The overall genital shedding rate was significantly higher in persons with symptomatic infection than with asymptomatic infection, 4753 of 23 683 days (20.1%; 95% CI, 18.3%-22.0%) vs 519 of 5070 days (10.2%; 95% CI, 7.7%-13.6%; $P<.001$). Genital HSV was detected at least once in 342 of 410 persons (83.4%; 95% CI, 79.8%-87.0%) with symptomatic HSV-2 infection and in 60 of 88 (68.2%; 95% CI, 58.5%-77.9%) persons with asymptomatic HSV-2 infection (FIGURE 1).

No genital lesions were reported on 25 664 days of follow-up (89.3% vs the model-predicted per-person rate of 87.9%; 95% CI, 86.3%-89.3%), and HSV was detected on 3142 (12.2%; 95% CI, 10.8%-13.5%) of those days. Subclinical genital shedding rates were higher in persons with symptomatic infection compared with asymptomatic infection (2708 of 20 735 [13.1%; 95% CI, 11.5%-14.6%] vs 434 of 4929 [8.8%; 95% CI, 6.3%-11.5%]; $P<.001$).

Genital lesions were reported on 3089 days with polymerase chain reaction swabs (10.7% vs model-predicted lesion rate of 12.1%; 95% CI, 10.7%-13.7%), including 2130 days (69.0%; 95% CI, 58.4%-72.7%) on which genital HSV was detected. During prospective follow-up, 262 (63.9%; 95% CI, 59.2%-68.5%) persons enrolled with symptomatic HSV-2 infection reported genital lesions at least once. In addition, 19 (21.6%; 95% CI, 13.0%-30.2%) persons who had asymptomatic, serologically diagnosed HSV-2 infection recognized lesions during the follow-up. The frequency of lesions during the study was higher among persons with symptomatic vs asymptomatic genital HSV-2 (3259 of 26 777 days [12.2% vs the model-predicted per-person rate of 13.9%; 95% CI, 12.2%-15.6%]) compared with 194 of 6336 days [3.1%; 95% CI, 2.3%-6.4%]; $P<.001$).

Overall, on days with detectable HSV, the median $\log_{10}$ copy number was 4.9 (IQR, 3.4-6.3). The median HSV $\log_{10}$ copy number was significantly higher among persons with symptomatic than asymptomatic infection, 4.9 (IQR, 3.5-6.3) vs 4.5 (IQR, 3.2-5.8), respectively ($P<.001$) (FIGURE 2). The median amount of HSV detected in the presence of lesions was also higher compared with the amount of HSV detected in the absence of lesions, 5.6 $\log_{10}$ (IQR, 4.3-6.7) vs 4.3 $\log_{10}$ (IQR, 3.1-5.6) copies/mL, respectively ($P<.001$) (Figure 2). However, the median amount of HSV detected during subclinical genital shedding episodes was similar in persons with symptomatic and asymptomatic infection (4.3 [IQR, 3.1-5.6] vs 4.2 [IQR, 2.9-5.5] $\log_{10}$ copies/mL; $P=.27$). Among persons with symptomatic genital herpes, 2708 (56.9%; 95% CI, 53.4%-59.6%) days of shedding were subclinical compared with 434 (83.6%; 95% CI, 69.4%-92.3%) days among persons with asymptomatic HSV-2 infection.

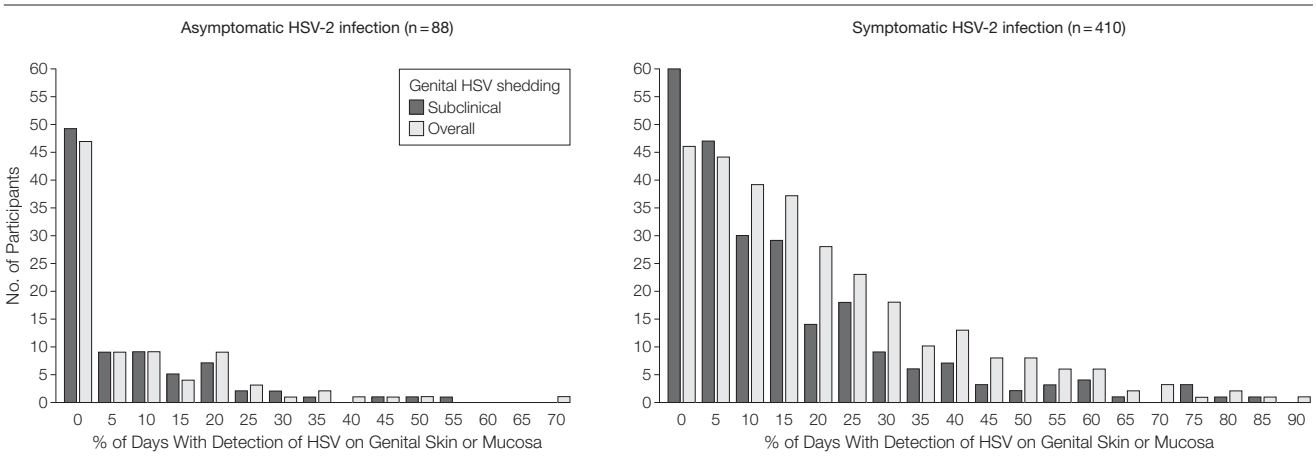
Shedding Episode Frequency and Duration

Viral shedding episodes associated with genital lesions were significantly longer than those without lesions (median 5.0 [IQR, 3.0-9.0] vs 2.0 [IQR, 1.0-3.5] days; $P<.001$). Persons with symptomatic infection had more frequent genital shedding episodes compared with persons with asymptomatic infection (median 17.9 [IQR, 11.9-27.1] vs 12.5 [IQR, 8.5-19.8] episodes per year; $P=.004$). In all persons, longer shedding episode duration was associated with higher maximum $\log_{10}$ copy number detected ($R^2=0.34$) (eFigure, available at http://www.jama.com).

Predictors of Genital Shedding

In univariate analyses, persons with asymptomatic HSV-2 infection had a shedding rate of 10.2% compared with a rate of 20.1% among persons with

Figure 1. Distribution of Genital Shedding Rate Among Asymptomatic and Symptomatic Infection Groups

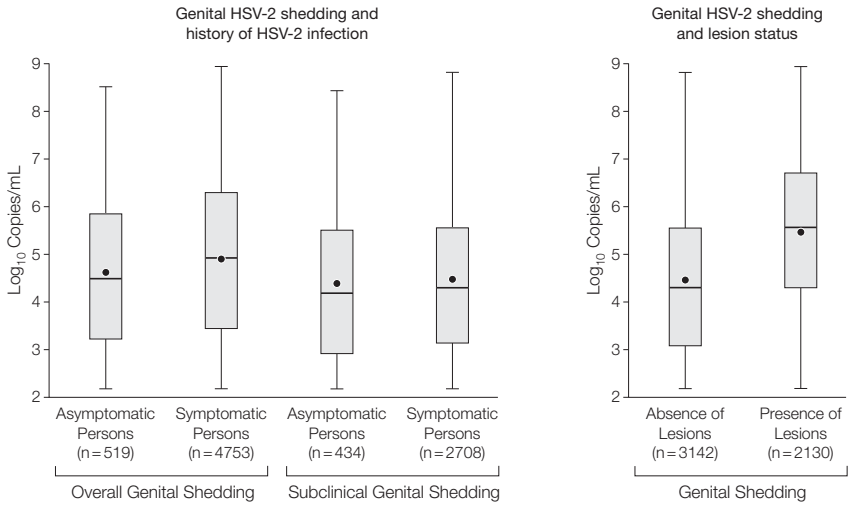


HSV-2 indicates herpes simplex virus type 2. Time intervals on the horizontal axes are equal to the value labeled on the tick and less than value of the next tick.

symptomatic genital herpes (risk ratio [RR] = 0.51; 95% CI, 0.38-0.68; $P < .001$) (TABLE 2). A higher number of recurrences per year was found to be a predictor of genital shedding. Persons with 8 or more recurrences per year had a shedding rate of 30.7% compared with 19.1% among persons with 1 to 7 recurrences per year (RR = 1.61; 95% CI, 1.27-2.03; $P < .001$). Persons of white race had a higher viral shedding rate than persons reporting nonwhite race (20.0% vs 10.8%; RR = 1.78; 95% CI, 1.22-2.60; $P = .003$). Herpes simplex virus type 1 infection did not influence the risk of HSV-2 genital shedding, with a shedding rate of 18.2% for persons with HSV-1 and HSV-2 vs 18.4% for persons with HSV-2 alone (RR = 0.99; 95% CI, 0.83-1.19; $P = .91$).

In the adjusted multivariate model, the risk for genital shedding remained lower among persons with asymptomatic HSV-2 infection compared with those with symptomatic infection (RR = 0.57; 95% CI, 0.42-0.77; $P < .001$). Higher number of recurrences per year and white race also remained predictive of higher genital shedding rate in the adjusted analysis (Table 2).

Figure 2. Herpes Simplex Virus Copy Number During Genital Shedding



Boxes represent interquartile range, dots represent means, whiskers represent the minimum and maximum, and horizontal lines represent the median. Numbers of swabs in each category are shown for each group. HSV-2 indicates herpes simplex virus type 2.

Predictors of Subclinical Genital Shedding

Asymptomatic infection was associated with lower rates of shedding in univariate analyses, with a shedding rate of 8.5% vs 12.9% among persons with symptomatic infection (RR = 0.66; 95% CI, 0.48-0.91; $P = .01$) (Table 2). Women had a slightly increased subclinical genital shedding rate compared with men, 13.2% of days vs 10.6% (RR = 1.25; 95% CI, 0.99-1.56; $P = .06$). Subclinical viral shedding was also more frequent among persons of white race compared with persons of other races, 12.9% vs 8.4% (RR = 1.53; 95% CI, 1.11-2.11; $P = .01$). Persons with 8 or greater recurrences per year had a viral shedding rate of 18.9% vs 12.7% among persons reporting 1 to 7 recurrences per year (RR = 1.49; 95% CI, 1.09-2.03; $P = .01$). HSV-1 infection did not influence the risk of subclinical HSV-2 genital shedding, 11.9% for persons with HSV-1 and HSV-2 vs 12.2% for HSV-2–only seropositive persons (RR = 0.98; 95% CI, 0.79-1.23; $P = .89$).

In the multivariate model, the risk for subclinical genital shedding among persons with asymptomatic HSV-2 infection remained lower compared with that of persons with symptomatic infection (RR = 0.72; 95% CI, 0.51-1.02; $P = .06$). White race and frequent recurrences also remained significant predictors of higher subclinical shedding (Table 2).

Predictors of Genital Lesions

In univariate analyses, persons with asymptomatic HSV-2 infection had lower rates of genital lesions compared with those with symptomatic HSV-2 infection, 3.8% vs 13.8%, respectively (RR = 0.28; 95% CI, 0.17-0.47; $P < .001$) (TABLE 3). No differences in lesion rates were observed by HSV-1 status, with lesion rates of 11.4% for persons infected with both HSV-1 and HSV-2 compared with 12.5% for persons with HSV-2 only (RR = 0.92; 95% CI, 0.72-1.18; $P = .50$). Persons of white race had a higher genital lesion rate compared with persons of other races, 13.3% vs 6.5% (RR = 2.06; 95% CI, 1.37-3.10; $P = .001$).

In the multivariate model, the risk for genital lesions among persons with asymptomatic HSV-2 infection remained lower compared with that of persons with symptomatic infection (RR = 0.27; 95% CI, 0.16-0.45; $P < .001$). Persons reporting white race also had a persistently higher genital lesion rate.

Recognition of Clinical Genital Herpes Among Asymptomatic Persons With HSV-2 Infection

Among the 88 persons who had asymptomatic HSV-2 infection, 19 persons reported genital signs and symptoms during follow-up. The participants who recognized lesions were slightly older than those who remained asymptomatic (mean age 45 years, SD 9.6 years vs mean age 42 years, SD 9.5 years; $P < .001$), but there were no differences in sex, race, or frequency of HSV-1 coinfection. The genital HSV shedding rate was 18.7% (227 of 1211 days) among persons in the asymptomatic group who reported genital lesions during the follow-up compared with 7.6% (292 of 3859 days) among the persons who

remained without any clinical manifestations of HSV-2 infection (RR = 2.47; 95% CI, 1.46-4.19; $P < .001$). The median $\log_{10}$ copy number among the participants who reported lesions was 4.8 (IQR, 3.7-6.6) compared with 4.1 (IQR, 2.9-5.4) among persons who remained asymptomatic ($P < .001$). Among persons in the asymptomatic group who reported genital lesion during follow-up, the subclinical viral shedding rate was 13.3% (142 of 1070 days) compared with 7.6% (292 of 3859) of days among persons who remained without any clinical manifestations of HSV-2 infection (RR = 1.86; 95% CI, 1.03-3.38; $P = .04$), but the copy number on subclinical shedding days was similar.

COMMENT

The uncertainty regarding the optimal management of asymptomatic persons who receive an HSV-2–seropositive test result derives in part from lack of information about the natural history of such infection. To address this gap, we prospectively evaluated a large cohort of HSV-2–seropositive persons to determine the patterns of genital viral shedding among persons with symptomatic and asymptomatic genital HSV-2 infection. We found that the risk for genital shedding was twice as high and the risk for lesions almost 3 times as high among persons with symptomatic genital HSV-2 infection. However, even among persons with asymptomatic HSV-2 infection, genital HSV shedding occurred on 10% of days, and almost all of it—84%—was subclinical. The quantity of virus (the median genital $\log_{10}$ copy number) shed subclinically was similar in persons with symptomatic and asymptomatic infection. Lesions were associated with a higher quantity of virus shed compared with that in subclinical shed-

Table 2. Predictors of Herpes Simplex Virus Type 2 Genital Shedding Rate

	Overall Genital Shedding Rate[a]				Subclinical Genital Shedding Rate[b]			
Parameter	Unadjusted Risk Ratio (95% CI)	Unadjusted *P* Value	Adjusted Risk Ratio (95% CI)	Adjusted *P* Value	Unadjusted Risk Ratio (95% CI)	Unadjusted *P* Value	Adjusted Risk Ratio (95% CI)	Adjusted *P* Value
History of infection of HSV-2								
Symptomatic	1 [Reference]	<.001	0.5 (0.42-0.77)	<.001	1 [Reference]	.01	0.72 (0.51-1.02)	.06
Asymptomatic	0.51 (0.38-0.68)				0.66 (0.48-0.91)			
Age, y								
≤30	1.67 (1.34-2.08)	<.001			1.88 (1.44-2.46)	<.001		
31-40	1.21 (0.98-1.50)	.08			1.15 (0.87-1.52)	.33		
≥41	1 [Reference]				1 [Reference]			
Unknown[c]	0.38 (0.24-0.60)	<.001			0.43 (0.26-0.71)	<.001		
Sex								
Male	1 [Reference]	.09			1 [Reference]	.06		
Female	1.17 (0.98-1.41)				1.25 (0.99-1.56)			
Race								
White	1.78 (1.22-2.60)	.003	1.74 (1.31-2.31)	<.001	1.53 (1.11-2.11)	.01	1.45 (1.05-2.00)	.03
Other	1 [Reference]				1 [Reference]			
Years since acquisition								
<1	2.20 (1.74-2.77)	<.001			3.06 (2.30-4.09)	<.001		
1-10	1.32 (1.07-1.63)	.01			1.48 (1.12-1.95)	.006		
11-40	1 [Reference]				1 [Reference]			
Unknown[d]	0.30 (0.33-0.41)	<.001			0.32 (0.24-0.43)	<.001		
Sexual orientation								
Men who have sex with men and bisexual men	1 [Reference]				1 [Reference]			
Heterosexual men[e]	1.37 (1.02-1.84)	.03			1.40 (0.97-2.02)	.07		
Women	1.41 (1.09-1.83)	<.001			1.51 (1.10-2.08)	.01		
HSV serology								
HSV-2 only	1 [Reference]	.91			1 [Reference]	.89		
HSV-1 and -2	0.99 (0.83-1.19)				0.98 (0.79-1.23)			
Recurrences per year								
1-7	1 [Reference]				1 [Reference]			
≥8	1.61 (1.27-2.03)	<.001	1.57 (1.25-1.97)	<.001	1.49 (1.09-2.03)	.01	1.46 (1.07-1.99)	.02
Unknown	0.77 (0.63-0.94)	<.001	0.96 (0.78-1.19)	.71	0.81 (0.63-1.03)	.09	0.94 (0.72-1.23)	.65

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2.
[a] The genital shedding rate was calculated per participant by using the number of days in which HSV DNA was detected divided by the total number of days with genital swabs.
[b] The subclinical genital shedding rate was calculated per participant by using the number of subclinical days in which HSV DNA was detected divided by the number of subclinical days with genital swabs.
[c] Age unknown for 41 persons.
[d] Years since acquisition unknown for 136 persons, including all 88 persons reporting asymptomatic acquisition.
[e] Thirty-four men with unknown sexual preference are categorized in this group.

ding. In addition, higher quantity of virus shed was associated with longer shedding duration.

These findings extend earlier observations that genital HSV shedding in persons who are seropositive for this virus is likely universal but that the clinical manifestations of disease differ widely.[5,8] Recent reports of $CD4^+$ and $CD8^+$ T-cell infiltrations into genital lesions that appear to persist for many months in the genital tract even in the absence of clinically evident ulcerations suggest that the immune system is actively surveilling the sites of HSV-2 reactivation.[19] However, the immune containment is imperfect, reflecting the inability of the immune response to control the reactivation, the almost constant release of herpes simplex virions from neuronal endings,[7] or both.

Despite the observation that biologically HSV-2 infection is best characterized along a continuum, we dichotomized our population into "symptomatic" and "asymptomatic" to mirror how the diagnosis is made in clinical practice: mostly on the basis of clinical presentation, but increasingly by type-specific serologic assays, which became commercially available in the United States in 1999. A similar dichotomy is often used in serologic surveys that assess whether HSV-2 antibody is associated with a previous diagnosis of genital herpes; usually only 10% to 25% of HSV-2–seropositive persons will have a history of genital herpes.[5,20,21] Further work is required to define to what extent this dichotomy reflects viral or host factors. However, even among persons with a history of genital HSV-2 infection, the spectrum of clinical disease is large, and our findings show that the virologic spectrum is also broad, with substantial overlap across the 2 groups. In addition, a substantial proportion of initially asymptomatic persons will recognize recurrent genital herpes once they receive a diagnosis and are educated,[5] as evidenced by the participants in this study with "asymptomatic" infection who observed genital lesions during the follow-up period. The lowest rates of viral shedding were among persons who remained asymptomatic throughout the study, although HSV-2 was still detected on more than 5% of days.

Table 3. Predictors of Herpes Simplex Virus Type 2 Genital Lesion Rate[a]

Parameter	Unadjusted Risk Ratio (95% CI)	Unadjusted *P* Value	Adjusted Risk Ratio (95% CI)	Adjusted *P* Value
History of infection of HSV-2				
Symptomatic	1 [Reference]	<.001	0.27 (0.16-0.45)	<.001
Asymptomatic	0.28 (0.17-0.47)			
Age, y				
<30	1.36 (0.99-1.86)	.06		
31-40	1.25 (0.94-1.66)	.13		
>41	1 [Reference]			
Unknown[b]	0.38 (0.19-0.75)	.006		
Sex				
Male	1 [Reference]	.92		
Female	0.99 (0.77-1.26)			
Race				
White	2.06 (1.37-3.10)	.001	2.15 (1.44-3.21)	<.001
Other	1 [Reference]			
Years since acquisition				
<1	1.18 (0.90-1.55)	.24		
1-10	1.24 (0.88-1.77)	.23		
11-40	1 [Reference]			
Unknown[c]	0.29 (0.18-0.46)	<.001		
Sexual orientation				
Men who have sex with men and bisexual men	1 [Reference]			
Heterosexual men[d]	1.42 (0.97-2.09)	.07		
Women	1.22 (0.86-1.72)	.27		
HSV serology				
HSV-2 only	1 [Reference]	.50		
HSV-1 and -2	0.92 (0.72-1.18)			

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2.
[a] The genital shedding rate was calculated per participant by using the number of days in which HSV DNA was detected divided by the total number of days with genital swabs.
[b] Age unknown for 41 persons.
[c] Years since acquisition was unknown for 136 persons, including all 88 persons reporting asymptomatic infection.
[d] Thirty-four men with unknown sexual orientation are categorized in this group.

Our study collected more than 28 000 genital swabs from 498 persons infected with HSV-2. Because the collection of genital swabs is time consuming, it is possible that participants who were concerned about genital HSV-2 infection were more likely to participate in this study. Because participants with asymptomatic HSV-2 infection were recruited in the absence of symptoms, it is unlikely that the asymptomatic cohort was inherently biased toward individuals with more severe HSV-2 infections. However, it is possible that a population-based study of genital herpes would have found milder disease among participants with a history of genital herpes. All genital swabs used for analysis were self-collected because we have shown previously that such swabs are as likely to yield virus as those collected by clinicians.[22] Our previous work has established that despite variations in the number of swabs collected by each participant, the shedding rates accurately represent virologic behavior.[8]

Our large cohort allowed us to detect several associations between demographic and clinical characteristics of the participants and the viral shedding. Several of the associations were small and their clinical relevance is uncertain. The similar shedding rate between men and women, both for overall and subclinical shedding, confirmed that men have subclinical shedding on normal-appearing genital skin. In addition, infection with HSV-1 does not

affect the frequency of HSV-2 reactivation, outside the early initial genital HSV-2 acquisition.[23] In this cohort, nonwhite persons had a lower viral shedding rate, but they represented a relatively small proportion of the cohort. Other studies have also suggested a differential response of African Americans to famciclovir.[24] As host genetic predictors of severity of HSV disease are identified, further work is needed to understand the potential for the gene-environment interactions in this infection.[25,26]

The finding that the quantity of virus shed during subclinical episodes is comparable in symptomatic and asymptomatic persons underscores the epidemiologic observations that the risk of HSV-2 transmission is high from persons with unrecognized HSV-2 infection. Most likely, there is a relationship between the risk of HSV-2 transmission and frequency and quantity of virus shed. In human immunodeficiency virus, in which this relationship has been characterized, the risk is higher at higher virus RNA loads, but the population that has very high viral load may be relatively small.[27,28] Thus, the risk of transmission is likely a function of both the frequency of shedding and the amount of virus present. The inoculum necessary for HSV-2 transmission to sexual partners is unknown, but neonatal HSV transmission,[29] as well as findings from the valacyclovir transmission trial,[30] suggest that even a quantitatively moderate shedding episode can result in transmission.[7]

Our findings suggest that "best practices" management of HSV-2–infected persons who learn that they are infected from serologic testing should include anticipatory guidance with regard to genital symptoms, as well as counseling about the potential for transmission. The issue of infectivity is both a patient management and a public health concern. The primary concern of many HSV-2–seropositive persons is the risk of transmission to sexual partners; in our experience this is the main source of angst in patients with genital herpes.[31] Several methods have been identified that partly reduce the risk of HSV-2 transmission to sexual partners. Condom use, daily valacyclovir therapy, and disclosure of HSV-2 serostatus each approximately halve the risk of HSV-2 transmission.[30,32,33] However, these approaches reach a small portion of the population and have not had an influence on HSV-2 seroprevalence in the last decade.[4] One of the reasons for such a limited effect is that few people are aware of their genital HSV-2 infection, and routine serologic testing, although available commercially, is recommended only in limited settings.[34] We hope that these data will result in further discussions regarding control programs for HSV-2 in the United States.

Author Contributions: Dr Wald had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Tronstein, Corey, Wald.
Acquisition of data: Huang, Warren, Corey, Wald.
Analysis and interpretation of data: Tronstein, Johnston, Selke, Magaret, Corey, Wald.
Drafting of the manuscript: Tronstein, Johnston, Selke, Wald.
Critical revision of the manuscript for important intellectual content: Tronstein, Johnston, Huang, Magaret, Warren, Corey, Wald.
Statistical analysis: Tronstein, Johnston, Selke, Magaret, Corey, Wald.
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Study supervision: Johnston, Corey, Wald.
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It is more important to know what kind of patient has the disease than what kind of disease the patient has.

—Sir William Osler (1849-1919)