CAUSES AND TREATMENT OF HERPES SIMPLEX VIRUS RECURRENCES

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ABSTRACT

CHRISTINA LUDEMA: Causes and treatment of herpes simplex virus recurrences
(Under the direction of Stephen R. Cole)

Trials have demonstrated that prophylactic acyclovir treatment reduces the cumulative risk of first herpes simplex virus (HSV) recurrence after randomization, and studies have suggested that ultraviolet (UV) exposure may increase the risk of HSV recurrence. We estimated the effect of acyclovir on cumulative risk of multiple recurrences and time outdoors on risk of ocular HSV recurrences. These goals were accomplished using the Herpetic Eye Disease study, a 12-month placebo-controlled randomized trial of twice-daily oral acyclovir to prevent ocular HSV recurrence.

Of the trial's 703 participants, 241 (34%) participants who started weekly report completion within 30 days of randomization were included in a nested study of orofacial herpetic recurrences. The estimated cumulative risk of non-ocular recurrence in the placebo and acyclovir groups after 180 days was 27% and 12% for first recurrences, and 11% and 3% for second recurrences, yielding risk differences of 15% (95% confidence interval (CI): 4, 26) and 8% (95% CI: 0, 15), respectively (homogeneity *P* value = 0.15). The unadjusted hazard ratios estimates for first and second recurrences were 0.51 (95% CI: 0.28, 0.92) and 0.55 (95% CI: 0.29, 1.03), respectively (homogeneity *P* value = 0.82); adjusted results were similar. Acyclovir was observed to have a smaller absolute effect and a similar relative effect on second as compared to first non-ocular HSV recurrence.

A total of 308 participants were included in a nested observational study that included reports of time spent outdoors. We matched weekly UV index values from the National Oceanic and Atmospheric Administration to each participant and used marginal structural Cox models to account for confounding due to time-varying psychological stress and contact lens use and selection bias from drop-out. The weighted hazard ratios comparing those with 8+ hours of time outdoors to those with less exposure were 0.84 (95% CI: 0.27, 2.63) and 3.10 (95% CI: 1.14, 8.48), for weeks with a UV index below 4, and 4+, respectively (ratio of hazard ratios: 3.68, 95% CI: 0.43, 31.4). Though imprecise, when UV index was higher (i.e. 4+), eight or more hours per week spent outdoors was associated with increased risk of ocular HSV recurrences.

To Sam, my rock
To Abby and Della, my joy
To Mom and Dad, my encouragement

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LIST OF ABBREVIATIONS

HEDS Herpetic Eye Disease study

HSV herpes simplex virus

NOAA National Oceanic and Atmospheric Administration

UV ultraviolet

Chapter I

CRITICAL REVIEW OF THE LITERATURE

1.1 Epidemiology of HSV

Infections with herpes simplex virus (HSV) are common; only 27% of the US population over the age of 12 is seronegative for both HSV-1 and HSV-2 (1, 2). Transmission of HSV-1 occurs primarily through nonsexual contact in early childhood or adolescence. By age 70, 90% of the population is infected with HSV-1 as seen in seroprevalence data and the high prevalence of HSV-1 in trigeminal ganglia of the general population (3-11). Acquisition of HSV-1 appears to be delayed with better sanitation practices, as age at seroconversion is generally higher in more developed countries (12). HSV-2 is usually transmitted through sexual contact or to infants by infected mothers. By age 70, 27% of the US population is seropositive for HSV-2 (3). Though HSV-1 has primarily been observed as an orofacial infection and HSV-2 as a primarily genital infection, the viruses can infect other sites and have with changing sexual practices (13-15). For example, a recent study done to assess the incidence of HSV infections among women negative at baseline, identified 2-times more genital infections caused by HSV-1 than HSV-2 (15).

1.2 Epidemiology of Herpetic Disease

After initial infection, HSV can be reactivated, resulting in recurring eruptive or inflammatory lesions. The consequences of recurrence vary by anatomical site. Ocular recurrences can lead to corneal scarring and loss of visual acuity (16). Orofacial and genital recurrences can result in painful lesions, and genital recurrences can increase susceptibility to co-infection with human immunodeficiency virus (16, 17). At any site, clinical HSV recurrences are associated with viral shedding (18). However, shedding does occur in the absence of symptoms (19), and some individuals never display symptoms of herpetic infections (15).

The age and sex adjusted incidence of symptomatic ocular HSV infections is estimated to be 11.8 per 100,000 person years (95% CL: 10.6, 13.0), and there is no difference in incidence between men and women, but incidence does increase with age with approximately 5 additional cases/100000 person years for each 10 year increase in age (20). Older estimates of ocular HSV incidence are shown in Table 1. After initial infection, an individual may develop recurrent disease, with a 10% a year risk of recurrence (20).

It is estimated that 20-40% of the general population suffers from herpes labialis (21). After primary oral infection with HSV-1, the mean rate of herpes labialis recurrence is 0.1 episodes per month (22). An estimated 40-60 million people are infected with HSV-2 in the US, with 600,000-800,000 clinical cases a year (23). Additionally, herpetic infections cause clinical disease at other sites including gingivostomastitis, skin infections, disseminated infection in infants, encephalitis, and other neurological conditions.

1.3 Pathobiology of HSV

HSV-1 and HSV-2 are neurotropic viruses; after initial infection at skin and mucosal surfaces, the virus migrates to neuronal cell bodies where it establishes latent infection. The strain of the virus, host genetics, and other factors are thought to affect whether an individual displays symptoms at initial infection (24). After initial infection, the virus travels to the sensory ganglia associated with the site of the initial infection (i.e., trigeminal ganglia for infections above the neck, sacral ganglia for infections below the neck) and establishes latency. Latency is defined as maintaining at least one functional viral genome in the absence of virus replication, assembly or pathologic effect. There is some evidence that herpes may additionally be able to establish latency in the cornea, though this question cannot be definitively answered until there are more sensitive HSV DNA tests (25). Reactivation leading to herpes virus production can be asymptomatic (i.e., shedding) or symptomatic (i.e., recurrence). In comparison to a primary infection, recurrences are often shorter in length with many of the symptoms (e.g., redness, pain, swelling) attributed to the host's immune response.

1.4 Causes of Recurrence

In animal models, social stress, hyperthermia, hypothermia, skin irritation, and exposure to UV light have been shown to cause recurrences (26). In humans, stress, fever, wind, UV exposure, and trauma are thought to be associated with HSV reactivation (27-32).

1.5 UV Exposure

There are many complex mechanisms through which UV exposure is thought to impact HSV recurrences; we will review two primary mechanisms hypothesized from animal models and cell culture. The first pathway is a direct effect of UV on HSV reactivation. Initially, UV radiation damages the ends of a nerve containing latent HSV inducing cell repair pathways. Cell repair, through c-Jun and c-Fos, activates the HSV transcription promoter (i.e., ICP0) leading to HSV transcription and reactivation (33). Additionally, these repair pathways circumvent the activity of HSV latency-associated transcript to prevent infected neurons from apoptosis (34), reactivating HSV.

The second pathway is through immune response depression due to UV exposure. UV radiation has been shown to suppress HSV antigen presentation in epidermal cells (35). UV is also likely to lead to the reduction of type 1 cytokine release, an important part of immunological control for viruses (36). T regulatory cells are induced in the lymph nodes draining the irradiated site, which release immunosuppressive modulators when stimulated by an antigen (37-40). Garssen et al. extrapolated from an animal model that 100 minutes of sunlight around noon on a clear summer day in southern Mediterranean countries would lead to a 50% suppression in the T cell response to a microbe (41). This localized immunosuppression may allow sufficient viral replication to cause a recurrence.

Some early studies of ocular HSV disease reported a seasonal peak during the winter months (42-45), and others found no seasonal association (43, 46, 47) (Table

2). Laboratory exposures to UV-B radiation have been used to induce recurrences within 1 to 7 days of exposure (27); however, some individuals were more susceptible to UV radiation than others (48). There has been one study and one abstract examining the relationship between time spent outdoors (i.e., at UV exposures that are reflective of daily life) and HSV recurrences.

The Herpetic Eye Disease Study was a randomized trial that enrolled 703 participants to assess the effect of prophylactic acyclovir on prevention of ocular herpes recurrences (49). As part of a nested cohort, 308 participants filled out weekly logs on various exposures thought to cause recurrences (50). Comparing those who spent ≥21 hours outdoors compared with those who spent <21 hours outdoors, the ocular HSV recurrence rate ratio was 1.93 (95% CL: 0.68, 5.49). There were 4 events and 40 events during weeks where participants spent ≥21 and <21 hours outdoors; therefore precision was not optimal for this endpoint. Without accounting for ambient UV, the effect of UV on ocular HSV recurence is likely diluted by time spent outdoors when UV index was low. Additionally, there was no accounting for time-varying confounding.

Wilhelmus and Todaro followed 28 participants in Houston for a year to assess the relationship between sunlight and herpetic keratitis (51). They found that the average amount of time spent outdoors in a week preceding a recurrence was higher than the mean sunlight exposure over the entire year (506 versus 467 minutes). However, they did not report results stratified by ambient UV, or account for time-varying confounding.

1.6 Therapeutic Strategies for HSV Infection and Recurrence

Treatment strategy for ocular HSV disease usually consists of trifluorothymidine, a nucleoside analog that interferes with DNA replication, topical or oral acyclovir, and possible adjuvant therapy of topical interferon (26). In the cases of disease that are primarily caused by inflammatory response (i.e., stromal keratitis), topical corticosteroids and long-term prophylactic use of acyclovir is recommended (52). Acyclovir is an antiviral drug that is used widely to treat HSV infections. Acyclovir, after being converted to its active form by HSV, interferes with HSV DNA replication more strongly that cellular DNA replication (53). Despite widespread use, the incidence of acyclovir resistance is low, particularly in immunocompetent individuals (54-56). None of these treatment strategies have been found to reduce viral shedding (57).

A vaccine for HSV has not yet been rolled out despite serious development efforts due to difficulties primarily related to the inadequacy of animal HSV infection models and the virus's lifelong infection and non-engagement with the immune system during latency (58). In light of these and other difficulties, vaccine developers have been focusing on reducing shedding and morbidity rather than prevention of infection and conferring lifetime immunity.

Table 1.1 Estimates of the incidence of ocular herpes disease

Author, Year	Location	Year(s)	Population	Ocular HSV incidence [a] (95% CI)
Young, 2010	Olmstead County, MN	1976-2007	Geographic cohort, numerator from ICD-9 codes in linked medical records, denominator from census, age and sex adjusted to US white population in 2000	11.8 (10.6, 13.0) 9.2 (8.1, 10.3) [b]
Labetoulle, 2005	France	Sept-Dec 2002	Sample of ophthalmologists, extrapolated incidence per Dr to all Drs in France, denominator was population of France in 2002	13.2 (10.4, 15.9) [b]
Liesegang, 1989	Rochester, MN	1950-1982	Geographic cohort, numerator from chart review and codes in linked medical records, denominator from census, age and sex adjusted to US white population in 1980	8.4 (6.9, 9.9)
Mortensen, 1979	Funen county, Denmark	1976-1978	Geographic cohort, registration of patients through ophthalmologists, denominator from census	12 [b,c]
Ribarić, 1976	Croatia		Not enough information to determine methods, seems that incident herpetic keratitis was considered among children only	4.2-12.5 [b]

Abbreviation: CI, confidence interval; Dr, doctor

[a] Per 100,000 person years

[b] Keratitis only

[c] Included people with history of keratitis

Table 1.2 UV, sunlight, and seasonal exposures and HSV recurrence

Author, Year	Exposure	Site of recurrence	Study type	N	Result
Ichihashi, 2004	Self-reported cause of recurrence	HSV-1	Cohort	4295 HSV+, 2656 had recurrence	Sun was reported as cause of recurrence in 10.4% of the entire study population
Wilhelmus, 1992 [b]	Ambient UV combined with hours outdoors	Ocular [c]	Unknown	28	The average amount of time spent outdoors in the week previous to the recurrence was higher than the overall average, cloud cover was weakly related to recurrences
HEDS, 2000	Self-reported time outdoors	Ocular	Cohort nested in RCT	308	Comparing those who spent ≥21 hours outdoors compared with those who spent <21 hours outdoors, the ocular HSV recurrence rate ratio was 1.93 (95% CL: 0.68, 5.49)
Spruance, 1995	UV	Oral (lip)	Quasi-experimental	20	45%-70% of participants experienced recurrence after being exposed to UV radiation, development of lesions correlated to past susceptibility to sun-induced herpes labialis
Gamus, 1995	Season	Ocular	Cohort	541	Incidence of ocular attack was highest in January ($p<0.04$), when subgroup analyses were performed, men, and those with epithelial keratitis were found to have significant circannual rhythms

Author, Year	Exposure	Site of recurrence	Study type	N	Result
Brant, 1994	Season	Ocular	Case-control	44	No seasonal variation in first infection was observed (data not reported), but reported that highest incidence was observed in January
Ucchio, 1993	Season	Ocular	Cohort	101	First recurrences occurred most frequently in Dec-May, second and third recurrences did not exhibit much seasonal variation, but were few
Rooney, 1991	UV	Oral (lip)	Experimental	38	Patients were exposed to UV on a site of previous recurrence on the lip on two different occasions, one with use of sunscreen, and the other unprotected, during unprotected exposure ¾ developed lesions, while during exposure with sunscreen use none developed lesions
Liesegang, 1989	Season	Ocular	Cohort	122	No apparent seasonal trends in first infection or in recurrences
Bell, 1982	Season	Ocular	Cohort	141	Recurrences most likely Nov-Feb
Mortensen, 1979	Season	Ocular	Cohort	107 [a]	No apparent seasonal trends in occurrences of ocular HSV
Norn, 1970	Season	Ocular	Cohort	226 [a]	No apparent seasonal trends in occurrences of ocular HSV

Abbreviation: HSV, herpes simplex virus; UV, ultraviolet
[a] Included incident cases as well as recurrent ones
[b] Abstract
[c] Keratitis only

Chapter II

STATEMENT OF SPECIFIC AIMS

2.1 Statement of Specific Aims and Hypotheses

The overall goal of this study is to contribute to the knowledge about treatment and prevention of HSV recurrence in two specific ways. First, as outlined in Aim 1, to assess the effect of acyclovir on HSV recurrences subsequent to the first after randomization. Second, as outlined in Aim 2, to estimate the effect of UV exposure on risk of ocular HSV recurrences.

2.1.1 Specific Aim 1

Our first specific aim is to estimate the effect of acyclovir on non-ocular HSV recurrences beyond the first recurrence after randomization. We hypothesize that because it is possible that individuals experiencing subsequent HSV recurrences may be non-responsive to treatment with acyclovir, we hypothesized that the protective effect of acyclovir would be weaker for a second non-ocular HSV recurrence than for the first non-ocular HSV recurrence.

2.1.2 Specific Aim 2

Our second specific aim is to estimate the total effects of time varying unprotected time spent outdoors on risk of ocular HSV recurrence, explore whether total effects are modified by UV index. We hypothesize that increased unprotected time spent outdoors will exhibit a positive dose-response association with HSV recurrence; will be modified (i.e., stronger) on days with a higher UV index.

2.2 Significance

Aim 1 examines the effect of randomized, prophylactic acyclovir on first and subsequent non-ocular recurrences (primarily orofacial). While the effect of prophylactic acyclovir on first recurrence post-randomization has been researched for ocular (49), orofacial (59-61), and genital herpes (62-67), no studies have looked at its effect on recurrences subsequent to the first. The experience of the patient does not end with a first recurrence after starting prophylactic acyclovir, so research is needed to inform best clinical practice.

Aim 2 quantifies the association of time spent outdoors with recurrence of clinical ocular and nonocular HSV infections. The literature for an association between sunlight exposure and HSV recurrence includes a wide range of effect estimates. Spruance et al. exposed people with oral HSV to ultraviolet (UV) radiation and observed a high rate of recurrence approximately 1 to 7 days after UV exposure, however there was no control group (27).

The proposed research will contribute to and clarify existing knowledge on time spent outdoors and HSV recurrence. Assuming our hypotheses for Aim 1 and 2

are borne-out, resultant clinical advice will help infected individuals better understand risk of recurrence while on prophylactic acyclovir and reduce morbidity by a reduction in the rate of recurrent episodes. In addition to improved clinical guidance, environmental controls may be feasible if a clear association between sunlight exposure and HSV recurrence is established (e.g., sunglasses designed to block critical UV wavelengths or UV blocking eye drops).

2.3 Innovation

2.3.1 Time-varying Confounding

Regarding Aim 2, Standard analytic methods fail to estimate the total effect of possible triggers on HSV recurrence whether or not we attempt to control for confounding. We will use marginal structural models (68-70) which are quantitative methods that disentangle the effects of multiple time varying triggers for HSV recurrence using complex longitudinal data.

These methods have been used in other scientific fields to solve similar problems (71-75). Cook, Cole and Hennekens (72) used structural models to estimate the association of aspirin use on cardiovascular mortality in the Physicians' Health Study; the hazard ratio from a standard analysis was 1.00 while the hazard ratio from a structural model, which reduced bias due to measured time varying confounding, was 0.75. Cole et al (68) used structural models to estimate the association of antiviral therapy use on incident AIDS in the Multicenter AIDS Cohort study; the hazard ratio from a standard analysis was 0.98, while the hazard ratio from a structural model was 0.54. These novel methods can yield dramatic results.

2.3.2 Assessment

Regarding Aim 2, the HEDS group reported a rate ratio of 1.93 (95% CL: 0.68, 5.49) of ocular HSV recurrence comparing those with >21 self-reported hours of unprotected sun exposure to those with ≤21 hours in the week prior to recurrence, a potential positive, albeit imprecise, association. We will incorporate UV index data from NOAA to better UV light exposure.

Chapter III

METHODS

3.1 Study Population and Setting

3.1.2 The Herpetic Eye Disease Study: Acyclovir Prevention Trial

The HEDS Acyclovir Prevention Trial was conducted to assess the effect of prophylactic acyclovir on ocular herpes recurrences. Participants were recruited through contact with ophthalmology offices located near the coordinating centers. Eligible participants were at least 12 years old and had a documented history of ocular HSV recurrence in the prior year with no clinical symptoms or treatment in the prior month. Patients were excluded if they were receiving antiviral or immunosuppressive therapy or had a history of immune dysfunction, renal insufficiency, allergy or adverse reaction to acyclovir, or keratoplasty or keratorefractive surgery in the involved eye. Seven hundred and three immunocompetent participants aged 12 to 90 years were enrolled from 74 clinical sites in the United States between 1992 and 1996 (49).

A permuted-block design was used to assign patients in approximately equal numbers between the treatment group and placebo group. Treatment was 400 mg of acyclovir (Zovirax, GlaxoSmithKline, Research Triangle Park, NC), orally, twice daily for 12 months. In the event of an ocular HSV recurrence treatment was

continued and use of topical corticosteroid treatment was left to the discretion of the clinician. Patients and clinic personal were masked to the treatment assignments; data analysts and the monitoring committee were not masked.

Compliance was assessed by counting the number of capsules remaining in each bottle when it was returned. When the bottle was not returned, compliance was estimated by the participant's report and from medication cards they used to record when medication was taken.

3.1.2 RFS substudy

A subset of participants volunteered for a nested prospective substudy to assess potential triggers of ocular HSV recurrence (50). Three hundred and eight participants filled out weekly diaries about the number of hours spent outdoors, eye injury, illness, contact lens use, menstrual cycle, and stress. Participants stopped filling out weekly diaries when they had an ocular recurrence. The protocol and informed consent forms for the trial and substudy were approved by the institutional review boards at the participating sites, and all participants provided written informed consent.

3.1.3 Data Quality

All data in this study were managed by the Jaeb National Coordinating Center where the data were entered in duplicate by trained staff. A random, masked sample of forms was re-entered to assess accuracy.

3.2 NOAA Data

The UV index is defined as $I_{UV} = k \times \int_{250nm}^{400nm} E_\lambda \times s(\lambda) d\lambda$, where the integral is taken from 250 nanometers (nm) to 400 nm, E_λ is the solar spectral irradiance, s(λ) is the erythema reference action spectrum (76), which weights the ultraviolet irradiance to values that cause skin redness, and k is a constant. UV indices are predicted by a computer model that relates atmospheric ozone levels to UV incidence on the ground, forecasted cloud coverage, and elevation (77). Index values usually range from 0 to 10, though values as high as 17 have been measured in locations where ozone is depleted. While $s(\lambda)$ is a skin related measure, the same spectrum affects ocular tissue (although additional wavelengths detrimentally affect the retina) (78, 79).

Data on the daily UV index forecasts for at least one city per state are publicly available through NOAA beginning in January 1994. Additionally, validated data are available on daily weather variables including precipitation, cloud cover, minimum, maximum, and average temperature. Participants were assigned weekly averaged UV index values corresponding to the city closest to the clinical site they attended. For study dates prior to January 1994 (8% of weeks) the UV index was multiply imputed (80, 81) thirty times, assuming that the UV index values were missing at random given the month of the year, daily values of precipitation, minimum temperature, maximum temperature, average temperature, cloud cover, and ocular recurrences (82).

3.3 Statistical Methods for Chapter 4 (Aim 1)

I will address Aim 1, estimating the effect of acyclovir on recurrences after the first, in Chapter 4. The clinical trial and cohort substudy, eligibility, treatment, and data quality have been addressed. Here we concentrate on statistical analysis.

3.3.1 Methods for recurrent events

When analyzing recurrent events in randomized trials, there are two primary difficulties. First, one of the benefits of randomization is that the placebo group and the treatment group are exchangeable (i.e., you could give the treatment to the placebo group and vice versa and expect to see the same results) conditional on complete follow-up. To ensure that people who are ineligible to get an event are not included in your analysis (i.e., immortal person-time), you might include only people who have had a first event in your analysis for a second event (i.e., conditioning on a prior event). However, once you condition on a first event (which is a post-randomization variable), you no longer have the benefits of exchangeability conferred by randomization (83). Instead, you have an observational study of people who have already had a first event.

Second, bias is introduced by conditioning on a first recurrence. To see this more clearly, a directed acyclic graph (84) is provided (Figure 3.1). Randomized exposure is represented here by X, so there are no causes that affect X; R1 and R2 represent first and second recurrences, respectively, and U represent unmeasured factors that are causes of recurrence. From this DAG, we can see that if you condition on X1, a path from X to R2 through U is opened by conditioning in a

common descendant of U and X. I consider traditional methods for analyzing recurrent events in light of these difficulties.

Recurrent events can be analyzed using a counting process approaches like the method described by Andersen and Gill (85). The rate of recurrent events, $d\mu(t)$, can be modeled with a proportional rate model $d\mu(t) = d\mu_0(t)\exp(\beta X)$. However, we were interested in comparing the effect of acyclovir on a second or later recurrence compared with the first, and so we needed methods that estimated risks of each recurrence separately.

Another method, described by Prentice, Williams, and Petersen (86) is to model the time between events, meaning the model for the kth event is conditional on the occurrence of the $k-1$th event. The hazard for this model is $h(t) = h_{0s}(t)\exp(\alpha X)$, where h_{0s} is the unknown, recurrence (s) specific baseline hazard. This formulation of the model estimates a common effect across recurrences, but a recurrence-specific effect can be estimated if $\alpha = \alpha_s$. This approach, however, does condition on a previous recurrence breaking randomization as discussed above. Additionally, as Wei (87) and Ghosh (83) note, this model implicitly assumes that the hazard time for the $k+1$th event is independent of the kth event.

Another approach was proposed by Wei, Lin, and Wessfeld which models each recurrent event separately (87). The hazard for the kth event is $h_k(t|x) = h_{0k}(t)\exp(\gamma x)$, where $h_{0k}(t)$ is the unspecified baseline hazard and $k = 1, \dots, K$ indexes the number of recurrences. As above, this model can be modified to allow recurrence-specific treatment effects by indexing γ by k. This model allows valid estimates of treatment effect past the first, and was the approach that we took

to model HSV recurrences. We counted person-time from the randomization day until each recurrence (i.e., first or second) or censoring (87). Therefore, participants contributed time at risk for the second HSV recurrence even if they did not have a first HSV nonocular recurrence. While this approach counts immune person-time (88), it provides a valid assessment of the effect of randomized treatment on multiple recurrence times as discussed by Wei et al (87), Ghosh (83), and Therneau et al (89).

3.3.2 Additional methods for Chapter 4

Participants were followed from randomization until drop-out from the study or 12 months later, which was the end of prophylactic acyclovir treatment. Participants were considered right censored if they dropped out or after 30 days of missing self-report diaries.

We used the complement of the Kaplan-Meier survival curve estimator (90) to estimate the cumulative risk of HSV recurrence. As a measure of absolute effect, we estimated risk differences by the difference in Kaplan-Meier estimates of risk between the two groups. Variance of the estimated risk difference was calculated by summing the Greenwood variance estimates from the two groups.

The number of persons who would require acyclovir treatment to reduce the recurrence caseload by one case, or number needed to treat (NNT), was calculated as the reciprocal of the estimated risk difference.

We also provide rates of first and second recurrence for both treatment groups, calculated as the number of HSV recurrences divided by the person-time

accumulated since randomization. We used the Poisson approximation to estimate the variance of rates (91).

As a measure of relative effect, we estimated the hazard of non-ocular recurrence for the acyclovir group relative to the placebo group. Specifically, we fit a Cox proportional hazards model using Efron's method for handling ties (92). The hazard at time t for recurrence k, for k = 1, 2 was modeled as $h_k(t|x) = h_{0k}(t)\exp\{\beta_k x\}$, where x = 1 if assigned acyclovir and 0 otherwise, $h_{0k}(t)$ is a recurrence-specific reference hazard function (93), and $\exp(\beta_k)$ is the relative hazard comparing those assigned to acyclovir to those assigned placebo. To account for possible bias introduced by analysis of a subsample of the randomized trial, we also present results adjusted for measured variables that were thought to be predictive of recurrence or predictive of being in the subsample, including gender, race or ethnic group (as white versus other), the categorized number of prior non-ocular recurrences (as: 0, 1-10, and 11+), and age at randomization (49). Age was modeled using a restricted quadratic spline with five knots placed at the 17th, 33rd, 50th, 67th, and 83rd percentiles, which were 31, 40, 42, 54, and 66 years (94) to create a smooth piecewise polynomial that allows for a flexible association between age and the hazard of non-ocular HSV recurrence. The spline can be thought of as a flexible line drawn through the averages of the y-values (in this case, the log hazard of non-ocular HSV recurrence) for a narrow group of consecutive ages on the x-axis.

When combining first and second recurrences, we used a robust variance estimator (87) to account for the dependence caused by the same participant providing information on more than one HSV recurrence.

The proportional hazards assumption was assessed graphically by plotting non-parametric estimates of the log cumulative hazard function computed separately for each arm. We also tested the proportional hazards assumption by including an interaction term of treatment group and time.

3.4 Statistical Methods for Chapter 5 (Aim 2)

3.4.1 Marginal Structural Models

A marginal structural pooled logistic regression model (68, 95, 96) is defined as $\log\{P(Y_{ij}^{\bar{x},\bar{c}=0}=1)/[1-P(Y_{ij}^{\bar{x},\bar{c}=0}=1)]\}=b_{0j}+b_1 g(\bar{x})$, where $Y_{ij}^{\bar{x},\bar{c}=0}$ is the time varying potential outcome of an indicator of HSV recurrence over the visit interval $(j, j+1]$. We estimate $b=\{b_{0j}, b_1\}$ as $\beta=\{\beta_{0j}, \beta_1\}$ by maximizing a weighted version of the Bernoulli likelihood function $L(\beta)=\prod_{i=1}^{N}\prod_{j=0}^{J-1} p_{ij}^{Y_i \widehat{W}_i} \times (1-p_{ij})^{(1-Y_i)\widehat{W}_i}$, where $p_{ij}=1/\{1+exp\left[-\left(\beta_{0j}+\beta_1 g(\bar{x})\right)\right]$.

Then the (discrete-time) hazard ratio is given as $\exp(\beta_1)$. Marginal structural models do not adjust for confounding by including covariates in the model, but adjust for confounding by weighting subjects according to the inverse of their probability of receiving exposure. The result is that such methods provide valid estimates of exposure effects even when time varying covariates act as both confounders and are affected by prior exposure.

Given the measured covariate histories, subject-specific inverse probability of treatment/exposure weights (henceforth, weights) are constructed as $W_{ij}=\prod_{m=0}^{j}\frac{f(X_{im}|\bar{X}_{im-1},\bar{C}_{im}=0,T_i>t_{im})}{f(X_{im}|\bar{X}_{im-1},\bar{C}_{im}=0,T_i>t_{im},\bar{Z}_{im-1})}$, where $C_{ij}=1$ if the participant is lost to

follow up between visit j and$j + 1$. The denominator of these weights can be understood as the probability that a participant received their own observed exposure given their past exposure and covariate histories and continued study participation. The numerator is the probability that the participant received their observed exposure conditional only on their past exposure history and continued study participation. We augmented our weights with an inverse probability-of-censoring weight that has a similar structure to this weight but instead uses covariates to predict the probability of not dropping out of the study.

3.4.2 Additional Methods for Chapter 5

Observed data were weighted by the product of stabilized inverse probability-of-exposure-and-censoring weights to account for confounding and selection bias by measured characteristics.

Time spent outdoors was dichotomized into two groups: 0 to 7 hours and 8+ hours due to the relatively small number of ocular recurrences. According to World Health Organization, reporting guidelines, UV index values of 2 or less, 3-5, 6-7, 8-10, and 11+ are considered low, moderate, high, very high, and extreme risk, respectively. Protection, including long sleeves, sunglasses and a hat are recommended for days when the UV index is 3+, and sunscreen is recommended for days when the UV index is 6+ (77). The median UV index the week prior to an ocular HSV recurrence was 4. We dichotomized the UV index at 4 to minimize variance.

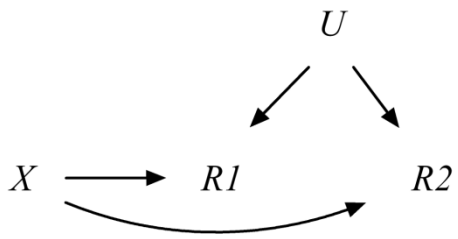
Hazard ratios were estimated using Cox proportional hazard models (97) with time on study as the time scale, and Efron's approximation for tied event times

(92). Inferences from all 30 multiple imputations were combined using Rubin's multiple imputation formula (80). Wald-type 95% confidence limits were estimated using the standard large-sample approximation for variance in the crude and weighted data, and the robust variance for weighted data (70).

To our knowledge, there is no established method for testing proportional hazards in the context of multiply-imputed covariates. A common method to test proportional hazards is to assess the slope (H_0=0, $P < 0.05$, Wald test) of a linear model fit to the Schoenfeld residuals (98). To account for the multiply-imputed data, we combined the slopes and variances of the Schoenfeld residuals over the 30 imputations using Rubin's multiple imputation formula (80).

Dichotomized hours outdoors and dropout were modeled using standard pooled logistic models (99). Covariates included age, psychological stress, UV index, gender, contact use, sunlight exposure history, and interaction terms between UV index and past sunlight exposures. UV index was not lagged; other time-varying covariates (i.e., stress, contact use) were lagged one visit. Age was included in the model using restricted quadratic splines with 3 knots, at 39, 52, and 65 years (94). Weights were stabilized by sunlight exposure history.

Figure 3.1 DAG for recurrent events in a randomized trial. X is the exposure, and R1 and R2 are the first and second recurrent events, respectively. U represents unmeasured confounding.



Chapter IV

PROPHYLACTIC ORAL ACYCLOVIR AND MULTIPLE HERPES SIMPLEX VIRUS RECURRENCES

4.1 Introduction

Approximately 70% of the United States population is seropositive for herpes simplex virus (HSV) type 1 or 2 (1, 2). After initial infection, HSV can be reactivated, resulting in recurring eruptive or inflammatory lesions. The consequences of recurrence vary by anatomical site. Ocular recurrences can lead to corneal scarring and loss of visual acuity (16). Orofacial and genital recurrences can result in painful lesions, and genital recurrences can increase susceptibility to co-infection with human immunodeficiency virus (16, 17). At any site, clinical HSV recurrences are associated with viral shedding (18). Reducing the frequency of HSV recurrences benefits the individual and decreases the spread of HSV to the susceptible population (100).

Prophylactic acyclovir (or its prodrug valacyclovir) reduces the hazard of first post-randomization HSV recurrence at different anatomical sites, including the ocular surface (49), mouth and lips (59-61), and genitalia (62-67). In a multicenter trial, the Herpetic Eye Disease Study, the hazards of first ocular and non-ocular

clinical HSV recurrence during one year among the acyclovir group were 0.55 (95% confidence interval (CI): 0.41, 0.75) and 0.51 (95% CI: 0.38, 0.69) times that of the placebo group, respectively (49). However, we are unaware of published studies that explored the effect of acyclovir on second HSV recurrences.

We estimated the protective effect of acyclovir on the first and second non-ocular HSV recurrence after randomization in the Herpetic Eye Disease Study. Because it is possible that individuals experiencing subsequent HSV recurrences may be non-responsive to treatment with acyclovir, we hypothesized that the protective effect of acyclovir would be weaker for a second non-ocular HSV recurrence than for the first non-ocular HSV recurrence.

4.2 Methods

4.2.1 Herpetic Eye Disease Study

The Herpetic Eye Disease Study enrolled 703 immunocompetent participants aged 12 to 90 years from 74 clinical sites in the United States between 1994 and 1996 (49). Participants had a documented history of ocular HSV recurrence in the prior year with no clinical symptoms in the prior month. A subset of participants volunteered for a nested prospective substudy to assess potential triggers of ocular HSV recurrence (50). Within 30 days after randomization, 241 began filling out weekly diaries. Participants who enrolled in the substudy more than 30 days after randomization were excluded from analysis. Participants stopped filling out weekly diaries when they had an ocular recurrence. The protocol and informed consent

forms for the trial and substudy were approved by the institutional review boards at the participating sites, and all participants provided written informed consent.

4.2.2 Prophylactic Treatment with Acyclovir

Participants were randomized to 400 mg of oral acyclovir or placebo taken twice daily for 12 months (49). Treatment was assigned by permuted-block design with equal allocation. Assigned treatment was continued for the 12-month duration of the trial regardless of ocular or non-ocular HSV recurrences. Results below are based on an intent-to-treat analysis in the sense that participants were analyzed in their randomized groups, regardless of compliance with the assigned treatment.

4.2.3 Ascertainment of Non-Ocular HSV Recurrences

Positive self-report of non-ocular HSV recurrence is the primary endpoint for this study. Ocular recurrences were not considered as the primary endpoint because participants did not record ocular recurrences subsequent to the first. Participants in the substudy reported non-ocular HSV recurrences in weekly diaries that were not collected on all participants in the randomized trial. Diaries included a section on illnesses experienced in the prior week in which the participants indicated if they had a non-ocular recurrence. Non-ocular recurrences included any oral, facial, or genital lesions observed by the participant and were not necessarily clinically verified by trial personnel. If a non-ocular HSV recurrence was reported in a given week, we used the midpoint of the week as the date of recurrence and considered

any recurrence reported during the subsequent two weeks as a part of the same recurrence episode (101, 102).

4.2.4 Statistical Methods

Participants were followed from randomization until drop-out from the study or 12 months later, which was the end of prophylactic acyclovir treatment. Participants were considered right censored if they dropped out or after 30 days of missing self-report diaries. We counted person-time from the randomization day until each recurrence (i.e., first or second) or censoring (87). Therefore, participants contributed time at risk for the second HSV recurrence even if they did not have a first HSV nonocular recurrence. While this approach appears to count immune person-time (88), it provides a valid assessment of the effect of randomized treatment on multiple recurrence times as discussed by Wei et al (87), Ghosh (83), and Therneau et al (89).

The complement of the Kaplan-Meier survival curve estimator (90) was used to estimate the cumulative risk of HSV recurrence. As a measure of absolute effect, risk differences were estimated by the difference in Kaplan-Meier estimates of risk between the two groups. Variance of the estimated risk difference was calculated by summing the Greenwood variance estimates from the two groups. The number of persons who would require acyclovir treatment to reduce the recurrence caseload by one case, or number needed to treat (NNT), was calculated as the reciprocal of the estimated risk difference. We also provide rates of first and second recurrence

for both treatment groups, calculated as the number of HSV recurrences divided by the person-time accumulated since randomization. We used the Poisson approximation to estimate the variance of rates (91).

As a measure of relative effect, we estimated the hazard of non-ocular recurrence for the acyclovir group relative to the placebo group. Specifically, we fit a Cox proportional hazards model using Efron's method for handling ties (92). The hazard at time t for recurrence k, for k = 1, 2 was modeled as $h_k(t|x) = h_{0k}(t)\exp\{\beta_k x\}$, where x = 1 if assigned acyclovir and 0 otherwise, $h_{0k}(t)$ is a recurrence-specific reference hazard function (93), and $\exp(\beta_k)$ is the relative hazard comparing those assigned to acyclovir to those assigned placebo. To account for possible bias introduced by analysis of a subsample of the randomized trial, we also present results adjusted for measured variables that were thought to be predictive of recurrence or predictive of being in the subsample, including gender, race or ethnic group (as white versus other), the categorized number of prior non-ocular recurrences (as: 0, 1-10, and 11+), and age at randomization (49). Age was modeled using a restricted quadratic spline with five knots placed at the 17th, 33rd, 50th, 67th, and 83rd percentiles, which were 31, 40, 42, 54, and 66 years(94) to create a smooth piecewise polynomial that allows for a flexible association between age and the hazard of non-ocular HSV recurrence. The spline can be thought of as a flexible line drawn through the averages of the y-values (in this case, the log hazard of non-ocular HSV recurrence) for a narrow group of consecutive ages on the x-axis. When combining first and second recurrences, we used a robust variance estimator (87) to account for the dependence caused by the same participant providing

information on more than one HSV recurrence. The proportional hazards assumption was informally assessed graphically by plotting non-parametric estimates of the log cumulative hazard function computed separately for each arm. We also tested the proportional hazards assumption by including an interaction term of treatment group and time. All analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

4.3 Results

Eighty-six percent of the substudy participants were white and 49% were female. The median age at randomization was 49 years old (quartiles: 37, 63). The median number of ocular and non-ocular recurrences ever prior to study entry were three (quartiles: 1, 5) and one (quartiles: 0, 11), respectively; 49% of participants reported a prior orofacial HSV recurrence. No notable differences in baseline characteristics were seen in either the acyclovir and placebo substudy groups as compared with the larger randomized trial (Table 4.1).

During 165 person-years of follow up for 241 substudy participants between April 1993 and October 1997, 125 participants completed diaries for the 12-month follow up and 116 were censored due to 30 or more days without a diary. Of those 116, 24 had ocular recurrences, 12 were lost to follow up in the larger trial, two died, two started open-label antiviral treatment, and six discontinued the study due to perceived side effects. Among the 218 participants (91% of the 241) who completed the full 12-month course of treatment, 86% of participants in the

acyclovir group and 92% of participants in the placebo group were at least 80% compliant with their assigned treatment.

During follow up, 93 non-ocular recurrences were reported: 47 were first recurrences, 23 were second recurrences, 11 were third recurrences, seven were fourth recurrences, and five were fifth or greater recurrences. At the end of follow-up, 194 participants (80%) had not experienced any non-ocular recurrences.

The estimated cumulative risk is shown in Figure 4.1 of a first (panel A) and a second (panel B) non-ocular HSV recurrence by randomized group over follow up. By 180 days, the estimated risk of first recurrence was 27% and 12% in the placebo and acyclovir groups (Table 4.2), respectively (first risk difference = 15%, 95% CI: 4, 26, number needed to treat = 7). The estimated risk of second recurrence by 180 days was 11% and 3% in the placebo and acyclovir groups, respectively (second risk difference = 8%, 95% CI: 0, 15, number needed to treat = 13). There was a notable difference between the risk difference estimates (difference of risk differences = 7%, 95% CI: -7, 20); a test of equality of the risk difference for first and second recurrences was not indicative of heterogeneity (*P* value = 0.30).

The rates of non-ocular recurrences in the placebo and acyclovir groups were, respectively, 45.3 (95% CI: 33.1, 57.5) and 22.8 (95% CI: 13.5, 32.0) recurrences per 100 person-years for the first recurrence, and 18.4 (95% CI: 9.7, 27.1) and 10.6 (95% CI: 4.0, 17.1) recurrences per 100 person-years for the second recurrence. The amount of person-time at risk of a first recurrence was smaller than the person-time at risk of a second recurrence because individuals with one or more

recurrences frequently contributed less time to the first recurrence analysis than to the second recurrence analysis (Table 4.3).

The unadjusted hazards of first and second recurrences in the acyclovir group were approximately half those in the placebo group (first HR = 0.51; 95% CI: 0.28, 0.92 and second HR = 0.55; 95% CI: 0.29, 1.0). Hazard ratios adjusted for sex, race or ethnic group, prior non-ocular recurrences, and age were similar to unadjusted results, albeit with slightly wider confidence intervals (Table 4.3). The ratio of first and second recurrence HRs is 0.93 (95% CI: 0.33, 2.06), indicating homogeneity. The summary HR was 0.52 (robust 95% CI: 0.28, 0.96), assuming the effect is the same across recurrences.

Visual inspection of the estimated log cumulative hazard function did not suggest a violation of the proportional hazards assumption (supplementary figure). The test for interaction between treatment group and time did not reject the null hypothesis for the first recurrence (*P* value for homogeneity = 0.32), but was suggestive of a departure from proportional hazards for the second recurrence (*P* value for homogeneity = 0.06). The hazard ratio for a second recurrence weakened over time. For instance, between randomization and 169 days (median of the case distribution for second recurrences) the hazard ratio for a second recurrence was 0.32 (95% CI: 0.09, 1.19), while the hazard ratio for a second recurrence between 170 and 365 days was 0.86 (95 % CI: 0.28, 2.67).

4.4 Discussion

We estimated the effect of acyclovir on first and second non-ocular recurrences, and our analysis mirrored results of the Herpetic Eye Disease Study of 703 participants (HR = 0.51; 95% confidence interval: 0.38, 0.69) for the effect of acyclovir on first non-ocular recurrences. Our analysis resulted in wider confidence intervals because we used a subsample of 241 participants with weekly data collected on multiple non-ocular HSV recurrences. While acyclovir did not prevent all recurrences, those in the acyclovir group experienced a lower rate of recurrence than those in the placebo group.

The estimated difference in the cumulative risk of first recurrence increases after three to four months (Figure 4.1, panel A), suggesting that some people may be more liable to experience HSV recurrence regardless of treatment, and after these participants have a recurrence in the first three to four months the effect of treatment is revealed in the remaining participants who are at lower risk for HSV recurrence. The hazard ratio for a second recurrence after 169 days (median of the case distribution for second recurrences) was closer to the null than during the first 169 days. While a decreasing biologic effect of acyclovir on second recurrences is possible, this result could also be the consequence of depletion of particularly susceptible individuals from the study over time (103).

We hypothesized that the hazard ratios for subsequent HSV recurrences would be weaker than the hazard ratio for the first HSV recurrence as participants experiencing subsequent HSV recurrences may be non-responsive to treatment with

acyclovir. We found a similar hazard ratio of first and second recurrences, but the absolute risk difference was smaller for second recurrences than for the first.

It should be noted that we examined the effect of acyclovir on first and second recurrences *after* randomization. These events could have been part of a long line of recurrences that a participant experienced or the first time a participant noticed an HSV infection at a specific site. Fifteen percent of participants (n=7) who experienced at least one non-ocular HSV recurrence during the study reported no non-ocular episodes before study entry. These participants may have had a primary infection with HSV that was analyzed as a recurrence, or they may not have noticed the lesion that accompanied initial infection.

Frequency of HSV recurrence is associated with serotype, viral genetics (104-106), recurrence site (22), and time since infection (107). This study population consisted of participants with ocular herpes that was most likely due to HSV-1. The recurrence rates that we found were similar to a randomized trial that reported rates of first genital herpes after randomization of 55 cases per 100 person-years in the placebo group and 30 cases per 100 person-years in the acyclovir group (108).

This study has several limitations. First, as the participants in this trial had recurring ocular HSV disease, the results of this study may or may not generalize to the population of individuals with recurring genital or orofacial HSV disease but without ocular HSV. Second, if participants experienced a non-ocular recurrence in the time between randomization and when they filled out their first diary up to one month later, those events would have been missed. Third, participants were censored when they had an ocular event. This censoring could have been

informative if those with ocular recurrences were more likely to also experience a non-ocular recurrence. However, the correlation between ocular and non-ocular recurrences was not strong. Fourth, there was notable loss to follow-up, with only 52% of participants completing their diaries through 12 months after randomization. Consider a scenario, in which a third of the population who was lost to follow-up in the acyclovir group had a non-ocular recurrence the day after the last diary followed by a second recurrence three weeks later (i.e., after the two-week "recovery" period), and all participants lost to follow up in the placebo group did not have a recurrence between loss to follow-up and the end of study at 12-months. Under this fairly extreme scenario, the hazard ratio for acyclovir versus placebo would be 1.1 for first recurrence and 1.7 for the second. Such an extreme case is unlikely but does illustrate the wide-ranging bounds under the observed loss to follow up. Fifth, non-ocular recurrences were not verified by a medical professional or laboratory testing and therefore may not have been actual HSV recurrences. Even so, the rates estimated are similar to a prior randomized trial with clinical verification of recurrences (108) suggesting that participants accurately reported their recurrences. Sixth, as the clinical trial was primarily focused on ocular recurrences, the anatomical sites for orofacial and genital herpetic recurrences were grouped together in study logs. Participants could have been reporting recurrences in other sites including genital and cutaneous sites; since 90% of first non-ocular recurrences that were reported in the full trial were orofacial (49), our results likely pertain primarily to herpetic episodes of the lips and mouth. Antibody testing could have provided an estimate of the number of participants co-infected with HSV-1 and

HSV-2, but those tests were not done in the trial. Finally, we were unable to investigate the effect of acyclovir on HSV recurrence subsequent to a second recurrence due to small numbers. Therefore, we do not know if the waning efficacy of acyclovir suggested on the absolute scale would also be observed in later recurrences.

A primary strength of this prospective cohort study is that it was nested in a randomized trial of acyclovir. As analyses were restricted to 241 of the 703 randomized participants who also participated in the substudy and filled out the weekly diaries, randomization may not have been preserved, and selection bias is possible. However, measured covariates remained balanced, adjusted results were similar to unadjusted results, and results for first non-ocular recurrence were similar to results in the larger trial, suggesting that minimal selection bias was induced by limiting analyses to the participants in the substudy. Furthermore, our statistical method allowed consistent estimation of hazard ratios for first and second recurrences (83, 87). This method creates risk sets for each recurrence from all participants in the substudy and permits participants to be included in the risk set for a second HSV recurrence despite not having experienced a first HSV recurrence. This strategy avoided the use of methods that may induce selection bias (109) by conditioning or stratifying on a prior recurrence (e.g., (86)). Estimating acyclovir's effect on second recurrences has public health importance since the experience of people with recurring HSV does not stop with the first recurrence after treatment initiation. These data suggest prophylactic treatment with acyclovir can be efficacious in preventing second recurrences. In conclusion, acyclovir halves

the hazard of recurrence for both first and second HSV recurrences and helps to prevent fewer second recurrences for which the event rate is lower.

Table 4.1 Characteristics of 703 Trial and 241 Substudy Participants Followed for HSV Recurrence Over 12 Months, United States, 1993-1997

Characteristic [a]	**Substudy Acyclovir Group (*n* = 122)**	**Substudy Placebo Group (*n* = 119)**	**Substudy** [b] **Both Groups (*n* = 241)**	**Trial Both Groups (*n* = 703)**
Age at randomization	48 (36, 61)	50 (37, 64)	49 (37, 63)	49 (35,64)
Female, % (*n*)	46 (56)	51 (61)	49 (117)	46 (326)
Race or ethnic group, % (*n*)				
White	84 (103)	87 (104)	86 (207)	79 (553)
Black	7 (9)	7 (8)	7 (17)	9 (65)
Other [c]	8 (10)	6 (7)	7 (17)	12 (85)
No. prior ocular recurrences	3 (1, 5)	3 (1, 5)	3 (1, 5)	3 (1, 5)
No. prior non-ocular recurrences	1 (0, 11)	1 (0, 11)	1 (0, 11)	1 (0, 11)
Type of prior non-ocular recurrences, % (*n*) [d]				
None	49 (60)	44 (52)	46 (112)	48 (338)
Orofacial	45 (55)	52 (62)	49 (117)	49 (347)
Genital	3 (4)	4 (5)	4 (9)	2 (14)
Other cutaneous sites	4 (5)	3 (3)	3 (8)	3 (23)

Abbreviation: No., Number

[a] Median (quartiles) unless noted otherwise

[b] Within 30 days after randomization, these participants enrolled in a substudy to assess potential triggers of ocular HSV recurrence

[c] Other includes those who self-reported as Asian, Hispanic, and other; the substudy population included 12 Asians and 5 Hispanics

[d] A participant could have experienced more than one type of prior recurrence

Table 4.2 Cumulative Risk of Non-ocular HSV by 180 Days in 241 Substudy Participants, United States, 1993-1997

HSV Recurrence No.	Treatment Group	Risk	95% CI	Risk Difference	95% CI	NNT
1	Placebo	0.27	0.17, 0.36	0		
	Acyclovir	0.12	0.06, 0.18	0.15	0.04, 0.26	7
2	Placebo	0.11	0.04, 0.17	0		
	Acyclovir	0.03	0.00, 0.06	0.08	0.00, 0.15	13

Abbreviation: CI, Confidence interval; No., Number; NNT, Number needed to treat

Table 4.3 Recurrence of Non-ocular HSV in 241 Substudy Participants, United States, 1993-1997

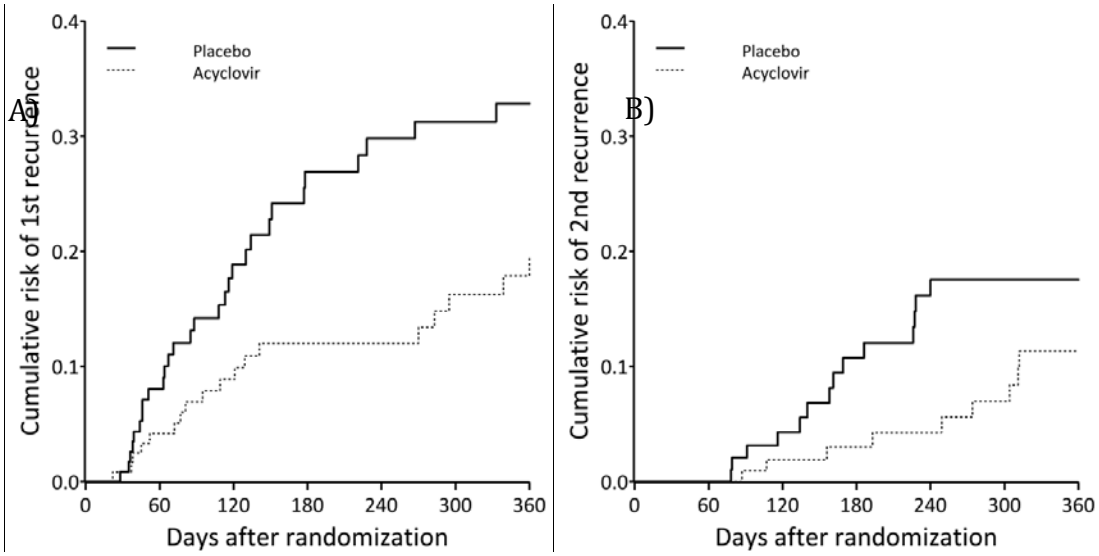
HSV Recurrence No.	Treatment Group	No. Events	No. Person-Years	Rate / 100 Person-Years	Crude Hazard Ratio	95% CI	Adjusted Hazard Ratio [a]	95% CI
1	Placebo	29	64	45.3	1		1	
	Acyclovir	18	79	22.8	0.51	0.28, 0.92	0.50	0.27, 0.94
	Total	47	143	32.9				
2	Placebo	14	76	18.4	1		1	
	Acyclovir	9	85	10.6	0.55	0.29, 1.03	0.54	0.27, 1.05
	Total	23	161	14.3				
Combined	Placebo	43	140	30.7	1		1	
	Acyclovir	27	164	16.5	0.52	0.28, 0.96 [b]	0.51	0.27, 0.96 [b]
	Total	70	304	23.0				

Abbreviation: CI, Confidence interval; No., Number

[a] Adjusted for sex, race or ethnic group, number of prior recurrences, and age

[b] Confidence intervals calculated using a robust sandwich variance estimator

Figure 4.1 Kaplan-Meier estimates of the cumulative risk of first (panel A) and second (panel B) non-ocular HSV recurrence for acyclovir treated (dashed) and placebo (solid) groups in 241 substudy participants.



Chapter V

ASSOCIATION BETWEEN UNPROTECTED UV EXPOSURE AND OCULAR HERPES SIMPLEX VIRUS RECURRENCE

5.1 Introduction

Approximately 70% of the US population is seropositive for herpes simplex virus (HSV) type 1 or 2 (3). After initial infection, HSV becomes latent in neuronal cell bodies, including those affecting the eye, but can be reactivated resulting in recurring eruptive lesions. Ocular HSV lesions can cause corneal scarring, and recurrent ocular HSV infections are a leading cause of visual loss (25, 47). Antiviral treatment is available for ocular HSV disease; in more severe cases physicians may recommend prophylactic treatment to prevent recurrences (26, 52). However, prophylaxis may not be entirely effective, making it essential to understand and intervene on causes of ocular recurrence.

Exposure to ultraviolet (UV)-B light has been used in animal models to induce HSV recurrences (110, 111), and to induce orofacial HSV recurrences in humans (27, 112). However, the existing literature regarding the association between season (42-46, 113, 114) or sunlight exposure (50, 51) and ocular HSV recurrence remains inadequate to inform preventive interventions related to limiting exposure to UV. Previous studies have largely based UV exposure on self-

reports (50), typically of the number of hours spent outdoors; however, this measure does not account for season- and weather-varying UV.

The Herpetic Eye Disease Study (HEDS) prospectively followed participants with ocular HSV for 15 months and found that participants exposed to >21 hours of sunlight per week experienced 1.93 times the rate of ocular HSV recurrence compared to those ≤21 hours, although results were imprecise with a nearly 10-fold wide CI (95% CI: 0.68, 5.49) (50). The HEDS analysis did not adequately control for confounding by time-varying factors, such as psychological stress. Psychological stress may be a time-varying confounder because psychological stress: 1) may be an independent predictor of recurrence (30) and, 2) may be affected by prior UV exposure (115-117). Moreover, there may be feedback between psychological stress and UV exposure given that the number of hours spent outdoors may be associated with the prior level of psychological stress. Simply using time-updated reports of sunlight exposure and including confounders in standard regression models does not allow for possible feedback between time-varying UV exposure and confounder levels (118). If there are time-varying confounders that are affected by prior sunlight exposure, then standard analytic methods (e.g., restriction, stratification, regression) fail to estimate the total (i.e., direct and indirect) effect of UV exposure on the risk of HSV recurrence. Regression adjustment blocks effects of UV exposure mediated through psychological stress, a time-varying confounder, as well as induces possible collider-conditioning bias (119). Marginal structural models can be used to obtain asymptotically consistent estimates of the total effect of UV exposure on ocular HSV recurrence in the presence of time-varying confounding (68-70).

We used weekly prospective data on self-reported hours of sunlight exposure, UV index measurements from the National Oceanic and Atmospheric Association, physician-documented HSV recurrences, and marginal structural models to estimate the total effect of unprotected UV exposure on ocular HSV recurrence.

5.2 Methods

5.2.1 Herpetic Eye Disease Study

The HEDS enrolled 703 individuals in a placebo-controlled randomized trial assessing the prophylactic effect of 500mg acyclovir orally twice-daily on ocular HSV recurrences (49). Participants were 12 years or older, immunocompetent, enrolled at 74 US clinical sites between 1992 and 1996, and had a documented history of ocular HSV recurrence in the prior year with no active disease in the month prior to study entry. Of 703 eligible participants, 308 volunteered to enroll in a nested cohort study to assess potential triggers of ocular HSV recurrence (50). Participants in this nested cohort study filled out weekly reports of the past week's exposures, including unprotected time outdoors and psychological stress. To avoid recall bias shown to be present in this cohort (120) and decrease misreporting, weekly forms that were completed more than 2 days before the end of the week, or more than 3 days after the end of the week (1850 forms), or that were missing the date of completion (320 forms) were excluded from this analysis. Participants were followed from study entry to first documented recurrence of ocular HSV, or censored at the end of study follow up (15 months) or at 3rd week of missing data

regardless of whether weekly reports were resumed (i.e., drop out).

The parent study protocol and informed consent forms were approved by the institutional review boards at the participating sites, and all participants provided written informed consent. Our analysis protocol was reviewed by the UNC Biomedical Institutional Review Board.

5.2.2 Ascertainment of HSV Recurrences

Ocular HSV recurrence was ascertained by a study-certified ophthalmologist using slit-lamp biomicroscopy at clinical examinations during months 1, 3, 6, 9, 12, 13, and 15 after randomization, and at additional times when participants attended the clinic reporting new ocular symptoms. Dates of recurrence were recorded, and analyses were conducted for first ocular HSV recurrence after enrollment.

5.2.3 Assessment of Sunlight Exposure, Covariates, and UV index

Weekly forms completed by participants were used to measure self-reported hours outdoors. Participants responded to the question "In the past week, approximately how many total hours did you spend outdoors unprotected by a brimmed hat or other protective clothing?", and responded in categories of 0 to 7, 8 to 14, 15 to 21, 22 to 28, or 29+ hours.

Age (in years), gender, race or ethnic group (self-reported, included in this study as white or other), study region, and numbers of self-reported prior ocular and non-ocular recurrences were collected at the first study visit. Weekly reports included information about contact use, regular use of glasses or sunglasses, and

psychological stress. Psychological stress was recorded by the participants weekly on the global stress rating scale (121), responding with 7-point Likert scale in answer to the question "How stressed did you feel in the past week?" As trial personnel collected data for the first trial visit, there were no missing data for time-fixed variables. Contact lens use (21% missing), psychological stress (25% missing), and time outdoors (24% missing) missing at baseline were set to study-wide median values; in the weeks following baseline, the previous week's covariate and exposure data were carried forward for a maximum of 3 weeks when weekly reports were missing. Of the 9,855 weeks after baseline included in the study, 20% was carried forward contact lens use, 21% was carried forward psychological stress, and 20% was carried forward time outdoors.

Data on the daily UV index for at least one city per state are publicly available through the National Oceanic and Atmospheric Administration beginning in January 1994. UV indices are predicted by a computer model that relates atmospheric ozone levels to UV incidence on the ground, forecasted cloud coverage, and elevation (77). Participants were assigned weekly averaged UV index values corresponding to the city closest to the clinical site they attended. For study dates prior to January 1994 (8% of weeks) the UV index was multiply imputed (80, 81) thirty times, assuming that the UV index values were missing at random given the month of the year, daily values of precipitation, minimum temperature, maximum temperature, average temperature, cloud cover, and ocular recurrences (82).

5.2.4 Statistical Analysis

Time spent outdoors was dichotomized into two groups: 0 to 7 hours per week and 8+ hours per week due to the relatively small number of ocular recurrences. According to World Health Organization, reporting guidelines, UV index values of 2 or less, 3-5, 6-7, 8-10, and 11+ are considered low, moderate, high, very high, and extreme risk, respectively. Protection, including long sleeves, sunglasses and a hat are recommended for days when the UV index is 3+, and sunscreen is recommended for days when the UV index is 6+ (77). The median UV index the week prior to an ocular HSV recurrence was 4; we dichotomized the UV index at 4 to improve precision.

Absolute incidence differences were estimated using additive Poisson regression (122). Hazard ratios were estimated using Cox proportional hazard models (97) with time on study as the time scale, and Efron's approximation for tied event times (92). Inferences from all 30 multiple imputations were combined using Rubin's multiple imputation formula (80). Wald-type 95% confidence limits were estimated using the standard large-sample approximation for variance in the crude models, and the robust variance for weighted models (70). The proportional hazards assumption was tested by computing the slope and variance of the Schoenfeld residuals and combining these values over the 30 imputations using Rubin's multiple imputation formula (80). A Wald test of the slope being equal to 0 (proportional hazards) was considered significant at the $P < 0.10$ level.

Observed data were weighted by the product of stabilized inverse probability-of-exposure-and-dropout weights to account for confounding and

selection bias by measured characteristics. Dichotomized hours outdoors and dropout were modeled using standard pooled logistic models (99). Candidate confounders included age, gender, race or ethnic group, number of prior ocular recurrences, number of prior non ocular recurrences, randomized assignment, psychological stress, glasses or sunglass use, and contact use. Randomized assignment was not considered as a confounder as it is not thought to be associated with time outdoors. Not included as confounders in the final weight model were race or ethnic group, number of prior ocular recurrences, number of prior non ocular recurrences, and glasses or sunglass use because addition of these variables did not alter the final effect estimate.

Time-fixed covariates included in the final model were age and gender. Age at baseline was included in the model using restricted quadratic splines with 3 knots, at 39, 52, and 65 years (94). Time-varying covariates included in the final model were psychological stress (lagged 1 week), UV index (not lagged), contact use (lagged 1 week), time spent outdoors (lagged 1, 2, and 3 weeks), and interaction terms between UV index and lagged outdoor exposures. Weights were stabilized by history of hours outdoors.

The resultant weights had a mean (SD) of 1.19 (4.03) with a range of 0.01 to 199.47. Inverse probability of exposure weights trimmed at 0.1 and 10 yielded weights with a mean (SD) of 1.01 (0.71). Results using the trimmed weights are reported here (95); use of the untrimmed weights produced similar results (as shown in Web Table 1). All analyses were conducted using SAS 9.3 (SAS Institute, Cary, North Carolina).

5.3 Results

Table 1 presents the data at study entry for 308 participants of the nested substudy, who were followed for a median of 29.5 weeks. At study entry, the participants had a median age of 49 years, 48% were female, and 85% self-reported as white. Compared to the entire population (n=308), those who had an ocular HSV recurrence were slightly older and less likely to have had self-reported non-ocular recurrences prior to study entry.

Table 2 presents data averaged over 10,163 ocular HSV recurrence-free person-weeks of follow up. In most of the person-weeks, observed participants reported 0-7 unprotected hours outdoors. Glasses and sunglass use were frequent, with 80% of participants using them on a regular basis. There were few differences between the weeks where participants reported 0-7 hours outdoors compared with 8+ hours outdoors. There were a total of 44 HSV recurrences, yielding an incidence of 4.3 events per 1000 person weeks. Of the 308 participants, 159 (52%) were administratively censored after more than 3 weeks of missing reports (Web Figure 1).

From our crude additive Poisson model, when comparing weeks with 0-7 and 8+ hours outdoors, we estimated an incidence difference of -0.50 cases per 1,000 person-years (95% CI: -2.01, 1.01) during weeks with a UV index <4, and 3.73 cases per 1,000 person-years (95% CI: 2.98, 4.48).

The weighted Kaplan-Meier curves of ocular HSV recurrence by UV index pooled over all 30 imputations, equivalent to taking the mean value over the

imputations, are shown in Figure 1. For the weighted analysis, in the weeks where the UV index was <4, the incidence of ocular HSV recurrence at 60 weeks was 23% for those exposed to 0-7 hours outdoors and 22% for 8+ hours. In the weeks where the UV index was 4+, the incidence of ocular HSV recurrence at 60 weeks was 13% for those exposed to 0-7 hours outdoors and 46% for 8+ hours.

Table 3 shows the estimates of the hazard ratio for ocular HSV recurrence due to sunlight exposure. Within levels of UV index exposure, we estimated crude hazard ratios of 0.81 (95% CI: 0.27, 2.41) and 2.42 (95% CI: 1.00, 5.84) for weeks with <4 UV index and 4+ UV index, respectively (ratio of hazard ratios: 2.99, 95% CI: 0.42, 21.4). The hazard ratios from a marginal structural Cox model that accounted for potential confounding by time-varying psychological stress and contact use, and baseline age and gender were 0.84 (95% CI: 0.27, 2.63) and 3.10 (95% CI: 1.14, 8.48), comparing 8+ hours outdoors to 0-7 hours, for weeks with <4 UV index and 4+ UV index, respectively (ratio of hazard ratios: 3.68, 95% CI: 0.43, 31.4).

The test of proportional hazards yielded *P*-values of 0.50 and 0.12 for the crude results and 0.47 and 0.10 for the inverse-probability-of-treatment-and-censoring weighted model, for <4 UV index and 4+ UV index respectively. The hazard ratio for ocular HSV recurrence weakened over time. For instance, between randomization and 29 weeks the marginal structural model hazard ratio of ocular HSV recurrence was 1.01 (95% CI: 0.14, 7.38) and 6.49 (95% CI: 1.40, 30.1) for weeks with <4 UV index and 4+ UV index, respectively, while the hazard ratio ocular HSV recurrence between 30 and 65 weeks was 0.78 (95% CI: 0.20, 3.12) and 1.41 (95% CI: 0.36, 5.52) for weeks with <4 UV index and 4+ UV index, respectively.

When restricted to the 155 participants who were randomized to receive acyclovir treatment (who experienced 23 events), similar patterns of crude hazard ratios comparing 8+ hours outdoors to 0-7 hours were observed. The crude hazard ratio for weeks with a <4 UV index was 0.74 (95% CI: 0.16, 3.36) and for weeks with 4+ UV index was 5.07 (95% CI: 1.34, 19.2). Results for the same groups for the weighted model were 1.10 (95% CI: 0.24, 5.02) and 7.57 (95% CI: 1.91, 30.1). Among the 153 who were randomized to receive placebo, results for the same groups for the crude and weighted models were 0.85 (95% CI: 0.16, 4.41) and 0.97 (95% CI: 0.26, 4.07), and 0.65 (95% CI: 0.11, 3.74) and 0.86 (95% CI: 0.18, 4.16), respectively.

5.4 Discussion

As the HEDS analysis did not appropriately adjust for time-varying psychological stress or contact lens use, we expected to observe a different hazard ratio of ocular HSV recurrence attributable to sunlight exposure. We found little evidence of time-varying confounding as evidenced by the similar effect estimates obtained from the crude and weighted models.

Similarly, we expected to see a stronger relationship between sunlight exposure and recurrence when UV index values were higher and by accounting for effect modification by UV exposure, we did find a stronger effect estimate of sunlight exposure on ocular HSV recurrences when the UV index value was 4+, however our results were imprecise. While we observed a high hazard of ocular recurrence particularly in the acyclovir group in weeks where the UV index was 4+, these

results rely and very few events, and are difficult to interpret in the absence similar effects observed in the literature.

There are many ways that UV exposure is thought to impact HSV recurrences; we review two mechanisms here. The first pathway is through immune response depression due to UV exposure. UV radiation has been shown to suppress HSV antigen presentation in epidermal cells (35), and lead to the reduction of type 1 cytokine release (36), an important part of immunological control for viruses such as HSV. Garssen et al. extrapolated from an animal model that 100 minutes of sunlight around noon on a clear summer day in southern Mediterranean countries would lead to a 50% suppression in the T cell response to a microbe (41). This localized immunosuppression may allow sufficient viral replication to cause a recurrence.

The second pathway by which UV radiation is thought to affect recurrence is directly through HSV reactivation. Initially, UV radiation damages the ends of a nerve containing latent HSV inducing cell repair pathways. Cell repair, through the c-Jun and c-Fos transcription factors, activates the HSV transcription promoter (ICP0) leading to HSV transcription and reactivation (33). Additionally, these repair pathways circumvent the activity of HSV latency-associated transcript (LAT) to prevent infected neurons from apoptosis (34), in turn, reactivating HSV.

Given the proposed mechanisms for UV light interaction with HSV recurrence, it becomes clear that looking for seasonal trends in recurrences or relating recurrences to time spent outdoors would likely not capture an individual's

exposure. While our assessment of UV exposure is more specific than previous studies, other methods (e.g., a UV dosimeter) would more finely delineate exposure.

There are limitations to this research. We assumed no unmeasured confounding and no informative censoring by unmeasured factors; if either of these assumptions were not met, our hazard ratios would be biased. In particular, psychological stress is a difficult quantity to reliably measure and may not be well captured in this study. Second, the results from this nested cohort may not generalize to a target population of those with ocular HSV disease that may differ in composition from trial populations (123, 124), often highly selective samples. Third, 51% of the participants were censored after not completing 3 weekly logs in a row. Fourth, while a fuller account of the covariate histories, a less restrictive functional form for age and time on study, and a broader set of covariates (e.g., region, systemic illness, acyclovir treatment) did not appreciably alter our results, there could have been model misspecification. Fifth, we assume positivity (125), that is a non-zero probability of exposure at every level of the observed confounders in the population.

Last, we assume consistency and its practical implication of exposure variation irrelevance (in this case, time spent outdoors) (126). This assumption is the most problematic here. Participants may have experienced their time spent outdoors differently (e.g. time spent outside in the early morning hours might be different from that during the afternoon). Additionally, an intervention to set individuals who usually spend 8+ hours outdoors to 0-7 hours would have to mimic the distribution of exposure found in the 0-7 hour group to see a similar effect

estimate to the one we estimated (127). However, we improved upon previous work by including UV index data to decrease unquantified exposure variation.

There are several strengths of this research. Ocular HSV recurrences were well documented and confirmed by study-trained physicians. The use of prospective data helps ensure the proper temporal order between exposures, confounders, and ocular HSV recurrences. Excluding weekly reports completed tardily minimizes recall bias (120). The use of time-updated reports reduces bias due to measurement error (128). Use of modern methods that allow feedback offers the opportunity to disentangle the relationship between UV and stress. Randomization to a certain number of hours spent outdoors may be difficult to implement and would likely have low compliance. Without randomized evidence, a thorough analysis of prospective observational data with repeated measures provides the best estimates of etiologic effects.

Findings from this and further research could guide clinicians in recommending behavioral interventions for those with ocular HSV. Physicians could recommend restriction on a patient's time outdoors during peak hours of UV exposure on days with high UV index. Another possible route for prevention of recurrence would be UV-blocking sunglasses, though it should be noted that many of the participants in this study did wear some type of glasses, so perhaps a brimmed hat or glasses with particularly strong UV protection should be considered.

In conclusion, we found an imprecise association between increased time spent outdoors and the risk of ocular HSV recurrences when UV index was relatively

high. Further research is needed both to replicate these results and to explore more finely UV exposure associated with increased risk of recurrence.

Table 5.1 Baseline characteristics of 308 total participants and 44 participants who experienced an ocular HSV recurrence, Herpetic Eye Disease Study, 1993-1997

Characteristic	All participants (*n*=308)			Ocular HSV recurrences (*n*=44)		
	No.	%	Median (IQR)	No.	%	Median (IQR)
Age at randomization (years)			49 (37, 63)			54 (38, 65)
Female	147	48		22	50	
Race or ethnic group						
White	263	85		41	93	
Black	20	6		1	2	
Other [a]	25	8		2	5	
Acyclovir treatment group	155	50		23	52	
Study center location						
New Orleans	33	11		5	11	
Houston	21	7		2	5	
San Francisco	61	20		5	11	
Atlanta	66	21		10	23	
Chicago	44	14		8	18	
Milwaukee	37	12		6	14	
Philadelphia	21	7		6	14	
New York	25	8		2	5	
No. prior ocular recurrences [b]			4 (1, 5)			3 (2 , 6)
No. prior non-ocular recurrences [b]			5 (0, 11)			0 (0, 9)

Abbreviations: No., Number; IQR, interquartile range

[a] Includes those who self-reported as Asian (18, 2) and Hispanic (7, 0) for all participants and cases, respectively

[b] Number of prior recurrences were self-reported at study enrollment

Table 5.2 Follow up characteristics of 10,480 person-weeks overall and stratified by self-reported sun exposure, Herpetic Eye Disease Study, 1993-1997

Characteristic	Follow up (*n*=10,163 person-weeks)			Sun 0-7 hours/week (*n*=7,169 person-weeks)			Sun 8+ hours/week (*n*=2,994 person-weeks)		
	No.	%	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)
Age at randomization (years)			52 (39, 65)			52 (40, 66)			49 (36, 63)
Female	4,822	47		3,616	50		1,206	40	
Race or ethnic group									
White	8,933	83		6,350	89		2,583	86	
Black	565	6		382	5		183	6	
Other [a]	665	6		437	6		228	8	
Acyclovir treatment group	5,337	53		3,708	52		1,629	54	
Study center location									
New Orleans	955	9		665	9		300	10	
Houston	755	7		616	9		139	5	
San Francisco	1,929	19		1,253	17		676	23	
Atlanta	1,878	18		1,383	19		495	17	
Chicago	1,560	15		1,046	15		514	17	
Milwaukee	1,525	15		1,062	15		463	15	
Philadelphia	697	7		517	7		180	6	
New York	864	9		637	9		227	8	
Current contact use [b]	1,024	10		787	11		237	8	
Glasses/sunglass use while outside [b]	8,037	79		5,631	79		2,406	80	
Overall stress [b, c]									
1 (lowest)	2,525	25		1,696	24		829	28	
2	2,073	20		1,424	20		649	22	
3	2,140	21		1,539	21		601	20	

4	1,667	16		1,210	17		457	15	
5	1,005	10		750	10		255	9	
6	493	5		350	5		143	5	
7 (highest)	260	3		200	3		60	2	
UV index			4.5 (2.4, 6.5)			3.9 (2.1, 6.2)			5.5 (3.3, 7.0)
Time spent outdoors [b]									
0 to 7 hours	7,169	71		7,169	100		0	0	
8 to 14 hours	1,742	17		0	0		1,742	58	
15 to 21 hours	668	7		0	0		668	22	
22 to 28 hours	265	3		0	0		265	9	
29 or more hours	319	3		0	0		319	11	

Abbreviations: No., Number; UV, ultraviolet; IQR, interquartile range

[a] Other includes those who self-reported as Asian (401, 237, 164) and Hispanic (281, 199, 82) for total follow up, Sun 0-7, and Sun 8+ respectively

[b] Before carrying forward data, the following amounts of data were missing (percentage of weeks prior to recurrence, drop-out or end of study period): contact use (20%), glasses/sunglass use (27%), overall stress (22%), time spent outdoors (20%)

[c] Stress was measured by asking the question "In the past week, how stressed have you felt overall" to which participants could answer on a seven-point Likert scale

Table 5.3 Estimated effect of sunlight and UV index exposure on time to HSV recurrence for 308 participants in the Herpetic Eye Disease Study, 1993-1997

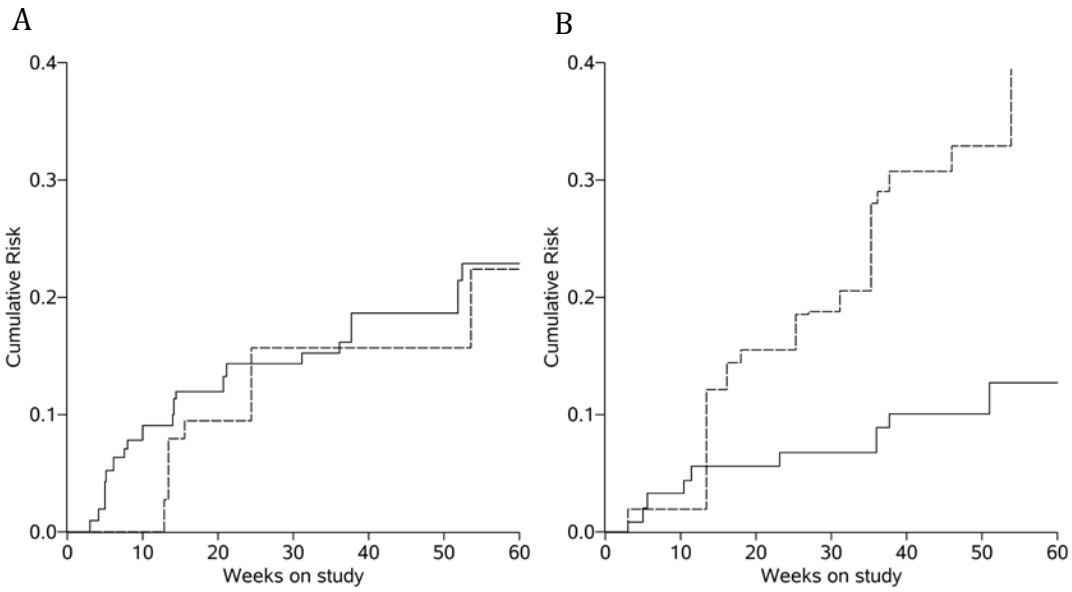
		No. of events	Person-weeks	Hazard Ratio	95% CI [a]
Crude	Sun ≤7 hours	27	7169	1.	
	Sun 8+ hours	17	2994	1.46	0.79, 2.68
	UV index <4				
	Sun ≤7 hours	18	3619	1.	
	Sun 8+ hours	4	949	0.81	0.27, 2.41
	UV index 4+				
	Sun ≤7 hours	9	3550	1.	
	Sun 8+ hours	13	2045	2.42	1.00, 5.84
Weighted [b]	Sun ≤7 hours	27	7169	1.	
	Sun 8+ hours	17	2994	1.85	0.89, 3.88
	UV index <4				
	Sun ≤7 hours	18	3619	1.	
	Sun 8+ hours	4	949	0.84	0.27, 2.63
	UV index 4+				
	Sun ≤7 hours	9	3550	1.	
	Sun 8+ hours	13	2045	3.10	1.14, 8.48

Abbreviations: No., Number; CI, confidence interval; UV, ultraviolet

[a] Robust variances, variances for results stratified by UV index account for variance between imputations

[b] Accounting for: time-varying psychological stress and contact use and baseline age and gender

Figure 5.1 Weighted Kaplan-Meier estimates of the cumulative risk of ocular HSV recurrence for person weeks with a UV index value <4 (panel A) and 4+ (panel B) for high sunlight exposure (dashed) and low sunlight exposure (solid) pooled over 30 imputations in 308 Herpetic Eye Disease Study participants



Chapter VI

DISCUSSION

6.1 Summary

The goal for this dissertation was to estimate the effects of acyclovir on orofacial recurrences and time outdoors on ocular HSV recurrences. This research was motivated by the burden HSV recurrences are to individuals, particularly ocular HSV recurrences which are a leading cause of infectious visual loss.

We obtained estimates of the effect of acyclovir on second orofacial recurrences. We noted that to maintain the benefits of randomization and to avoid collider-stratification bias, analyses that included immortal person-time were required. Acyclovir did have a similar relative effect on second recurrences when compared with first recurrences. We also analyzed the effect of time outdoors on ocular HSV recurrences using marginal structural models to account for time-varying confounding. We found an association, though imprecise, between time outdoors with ocular HSV recurrences when UV index was high.

6.2 Limitations

The HEDS trial was not intended to be a representative sample of those who have ocular HSV, or even those who experience ocular HSV recurrences. Participants

who take part in randomized trials may not be representative of these two target populations. Trial participants are more likely to have health-seeking behaviors, but also may be more likely to have serious disease. Additionally, there was a high rate of administrative censoring in our study due to weekly report completion. If the participants that remained under study were not representative of those who did not, this could cause selection bias in our results. Further study of these two exposures, prophylactic acyclovir and time spent outdoors, should be done in populations that are similar to the general population with HSV recurrences.

In both Chapters 4 and 5, we observed a decreasing effect of exposure (i.e., acyclovir and time outdoors) over time. While it is concedable that the effect of these exposures waned over time, depletion of susceptible participants in the trial over time is a possible explanation.

The primary limitation in Chapter 4 was the measurement of the outcome, non-ocular recurrences. Though it was most likely that the outcomes reported by the study participants were orofacial recurrences, this was not verified by a clinician nor was the site documented by the participant. A second limitation was the small number of third or later recurrences. A third limitation was the estimation of effects using only intent-to-treat analysis. Had the trial collected time-varying compliance data, we could have properly adjusted for compliance in an as-treated analysis (129).

The primary limitation of Chapter 5 was the assumption of consistency (130) and its practical implication of exposure variation irrelevance (131) (in this case, time spent outdoors). Participants may have experienced their time spent outdoors

differently (e.g. time spent outside in the early morning hours might be different from that during the afternoon). Additionally, an intervention to set individuals who usually spend 8+ hours outdoors to 0-7 hours would have to mimic the distribution of exposure found in the 0-7 hour group to see a similar effect estimate to the one we estimated. While we significantly improved on the measurement of UV compared to prior studies, matching UV values to a person's study center is not the ideal precise personal measurement.

6.3 Strengths

The primary strength of the results presented in Chapter 4 was that they were nested in a randomized trial of acyclovir. Though we did restrict to those who filled out weekly diaries, measured covariates remained balanced, adjusted results were similar to unadjusted results, and results for first non-ocular recurrence were similar to results in the larger trial, suggesting that minimal selection bias was induced by restricting to the substudy. We also used statistical methods to allow consistent estimation of hazard ratios for first and second recurrences.

In Chapter 5, ocular HSV recurrences were well documented and confirmed by study-trained physicians. The use of prospective data helped ensure the proper temporal order between exposures, confounders, and ocular HSV recurrences. Excluding weekly reports completed tardily minimized recall bias. The use of time-updated reports reduced bias due to measurement error. Use of modern methods that allow feedback offered the opportunity to disentangle the relationship between UV and stress.

6.4 Future Directions for Public Health Policy

As our findings in Chapters 4 and 5 do not build upon a large body of previous literature, further research should be undertaken before changes in public health policy should be advocated. That being said, upon replication of results similar to those found in Chapter 4 would suggest that individuals on prophylactic acyclovir can expect their treatment to prevent later recurrences as well as first ones so treatment should be continued.

Should results in Chapter 5 be replicated, individuals with frequently recurring ocular HSV may want to limit time outdoors when UV index is high. However, decisions made to restrict UV exposure should also be informed by the beneficial effects of sunlight exposure (e.g., vitamin D production, exercise). Our findings ought to be taken together with other literature on prevention of recurrence to provide a comprehensive picture for individuals to assess what kinds of pharmacologic or behavioral interventions would best suit their needs.

6.5 Future Directions for Research

As there have been continued difficulties in finding an effective HSV vaccine to prevent infection and confer lifetime immunity, the goal of understanding how to intervene upon recurrence continues to be relevant. Prophylactic acyclovir and other related antivirals are an essential treatment option for those who have recurrent disease. Additionally, there has been some evidence that high-dose valacyclovir (a prodrug of acyclovir) may reduce viral shedding and thus transmission. Moving forward, recurrences subsequent to the first after

randomization or study entry should be included in studies of high-dose prophylactic antivirals to be efficient with the data collected and to shed further light on the experience of individuals who experience breakthrough recurrences on treatment.

More research is needed on the relationship between UV exposure, time spent outdoors, and ocular HSV recurrences. In particular, better measures of UV exposure (i.e., dosimeters), or information about the time of day spent outdoors would improve causal inference from these studies. Equally critical is to assess whether protection such as sunglasses, UV blocking eye drops, and brimmed hats can effectively prevent recurrences while allowing individuals to participate in outdoor activities.

6.6 Conclusion

We found associations between acyclovir use and cumulative risk of first and second orofacial HSV recurrences and between time outdoors and ocular HSV recurrence when the UV index was high. Replication of this work is needed before policy recommendations are explored.

Appendix 1. DATA COLLECTION FORMS FROM THE HEDS.

HEDS Form 12.1 (8/25/94)
Page 1 of 3

HERPETIC EYE DISEASE STUDY
RFS Weekly Log

ID No.: ___ ___-___ ___-___ ___ ___	Week of: ___ ___-___ ___-___ ___ to ___ ___-___ ___-___ ___
	mo day yr mo day yr

Please fill out this form completely each Sunday night reporting events that happened during the past week (Monday through Sunday night).

Date Filled in: ___ ___-___ ___-___ ___
mo day yr

A. EYE PROBLEMS

Did you have any problems this week with your eye? Circle "NO" or "YES" for each question, or N/A if Not Applicable.			
eye injury at work?		No	Yes
eye injury at place other than work?		No	Yes
got something in eye?		No	Yes
contact lens irritation?	N/A	No	Yes
recurrence of herpes of the eye (ocular herpes)?		No	Yes
If YES to any of the above:			
a. Date it happened ___ ___/___ ___/___ ___ (mo day yr)			
b. Did you see an eye professional about it?		No	Yes
c. Explain what happened to your eye _____			

B. ILLNESSES

Did you have an illness this week? (circle one) "Do not include minor complaints that did not develop into illness, for example, a mild stomach ache."	No	Yes
If YES, check (√) off type of the illness(es) you had:		
_____ cold or respiratory infection (cough, runny or stuffy nose, sore throat)		
_____ stomach flu, vomiting, or diarrhea		
_____ allergies, hay fever		
_____ fever		
_____ asthma attack		
_____ urinary tract infection		
_____ rash or skin infection (please specify location and type): _____		
_____ non-ocular herpetic recurrences (please specify location, e.g. mouth/lip, genital): _____		
_____ other type of infection or problem (please specify): _____		

C. FOR PRE-MENOPAUSAL WOMEN ONLY

Did your menstrual period begin this week? (circle one)	No	Yes
If YES, on what date did it begin? __ __/__ __/__ __ (mo day yr)		

D. SUN EXPOSURE

In the past week, approximately how many total hours did you spend outdoors unprotected by a brimmed hat or other protective covering? (Don't count the times you were driving in a car, or when it was raining.) (circle one)

0-7 hours | 8-14 hours | 15-21 hours | 22-28 hours | 29 or more hours

During the past week, did you regularly wear sunglasses or regular glasses when outside? (circle one)	No	Yes

E. COMPLETE ONLY IF YOU ARE A CONTACT LENS WEARER

Did you wear contact lenses this week? (circle one)	No	Yes
If YES, did you sleep with your contacts in this week?	No	Yes

F. STRESSFUL SITUATIONS

Below please briefly describe any situations in your life that have been stressful for you FOR THE LAST TWO WEEKS OR LONGER. Even if you have listed them on previous weekly logs, please list them again here if they were stressful for you this week.

For EACH SITUATION, indicate how long it's been stressful, and rate its stressfulness in the past week. "1" indicates the situation was not at all stressful, "4" indicates it was moderately stressful, and "7" indicates it was extremely stressful. Use any number from 1 through 7 to indicate how stressful it was for you.

Situation (Briefly describe the stress situation)	For how long was this situation stressful (# weeks, months, years)	How stressful was this for you? (circle one for each) Not at all ... Moderately ... Extremely
1.)		1 2 3 4 5 6 7
2.)		1 2 3 4 5 6 7
3.)		1 2 3 4 5 6 7
4.)		1 2 3 4 5 6 7

On average, how stressful was it for you this past week in the following areas of your life?

Circle any number from 1 through 7 to show how stressful it was in each area, or if not applicable circle N/A. "1" indicates the situation was not at all stressful, "4" indicates it was moderately stressful, and "7" indicates it was extremely stressful. Circle an item as Not Applicable if it does not apply to you (for example, circle N/A for "work-related" if you don't work).

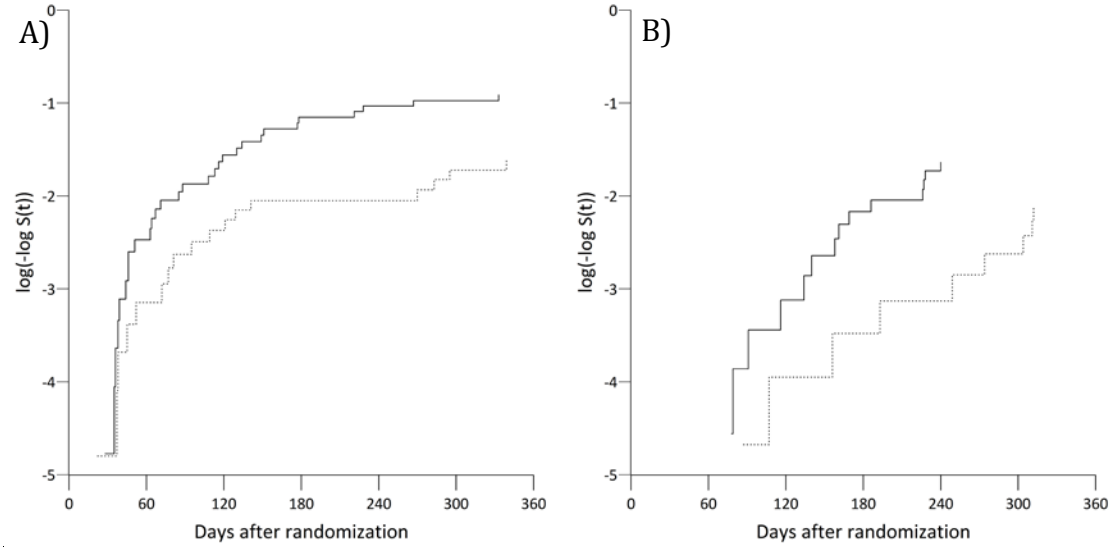
Be sure to circle a response on every line.

		How stressful was it for you? (circle one for each)						
	Not Applicable	Not at all			Moderately			Extremely
Your health		1	2	3	4	5	6	7
Financial		1	2	3	4	5	6	7
Home situation (home environment)		1	2	3	4	5	6	7
Personal goals		1	2	3	4	5	6	7
Relationships outside the home		1	2	3	4	5	6	7
Relationships within the home	N/A	1	2	3	4	5	6	7
Problems of friends or family (stressed because your friends or family have problems)	N/A	1	2	3	4	5	6	7
School-related	N/A	1	2	3	4	5	6	7
Work-related (paid employment)	N/A	1	2	3	4	5	6	7
Unemployment-related (if laid off or fired from your job)	N/A	1	2	3	4	5	6	7
Retirement-related	N/A	1	2	3	4	5	6	7
OVERALL, how stressed have you felt this past week?		1	2	3	4	5	6	7

IF YOU HAVE QUESTIONS ABOUT THIS FORM, CALL THE RFS COORDINATOR AT [1-800-388-HEDS]

Appendix 2. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 4

Figure A.2.1 Estimated log cumulative hazard functions for acyclovir treated (dashed) and placebo (solid) groups to non-ocular recurrence in days for first recurrence (panel A) and second recurrence (panel B).



Appendix 3. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 5

Table A.3.1 Adjusted hazard ratios using full weights and trimmed[a] weights

	Full	**Trimmed**
UV index <4	0.64	0.84
UV index 4+	2.8	3.1

[a] Inverse probability of exposure weights trimmed at 0.1 and 10

Table A.3.2 Rates of ocular HSV recurrences by time spent outdoors

	No. of events	**Person-weeks**	**Rate /1000 person weeks**
Sun ≤7 hours	27	7169	3.8
Sun 8+ hours	17	2994	5.7
UV index <4			
Sun ≤7 hours	18	3619	5.0
Sun 8+ hours	4	949	4.2
UV index 4+			
Sun ≤7 hours	9	3550	2.5
Sun 8+ hours	13	2045	6.4

Abbreviations: No., Number

Table A.3.3 Distribution of time spent outdoors by UV index

	UV index <4	**UV index 4+**
Sun ≤7 hours	3,633	3,536
Sun 8-14 hours	584	1,158
Sun 15- 21 hours	173	495
Sun 22-28 hours	81	184
Sun 19+ hours	105	214

Table A.3.4 Description of weights over time

	Mean	SD	Min	Max	1st Pctl	25th Pctl	50th Pctl	75th Pctl	99th Pctl
Untrimmed									
Exposure	1.18	3.833	0.005	184.151	0.199	0.714	0.941	1.099	3.468
Censoring	0.998	0.175	0.503	2.584	0.678	0.889	0.989	1.074	1.549
Combined	1.106	2.332	0.006	104.769	0.186	0.680	0.922	1.118	3.418
Trimmed									
Combined	1.007	0.671	0.089	13.483	0.186	0.680	0.922	1.118	3.418

Abbreviations: SD, Standard Deviation; Pctl, Percentile; Min, Minimum; Max, Maximum

Figure A.3.1 Number of HEDS participants over time on study. Dots represent ocular recurrences. Participants were censored from the study population at the time of ocular recurrence or after 3 weeks of missing weekly reports.

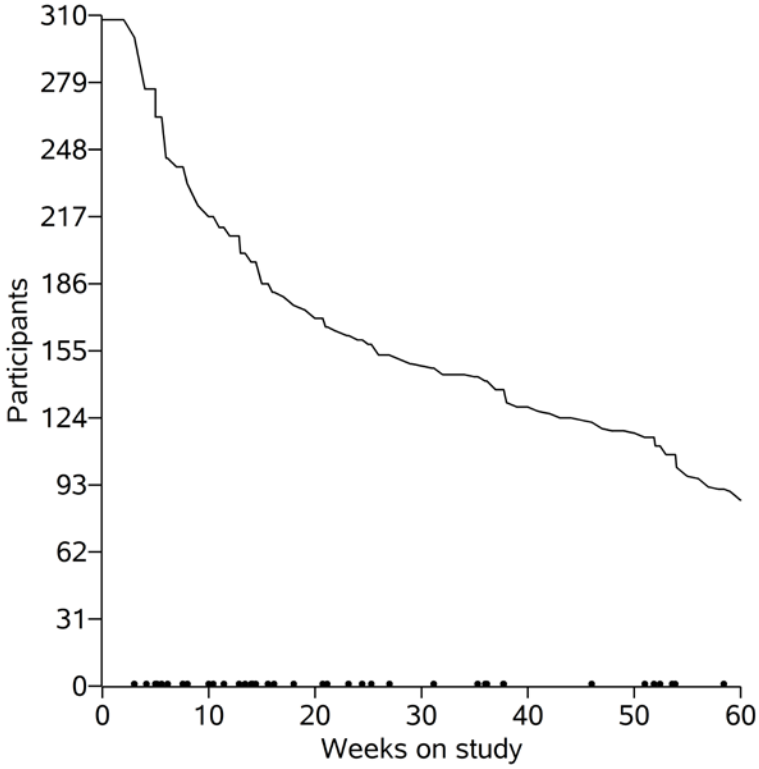


Figure A.3.2 Proportion of participants who spent 8+ hours outdoors by time on study.

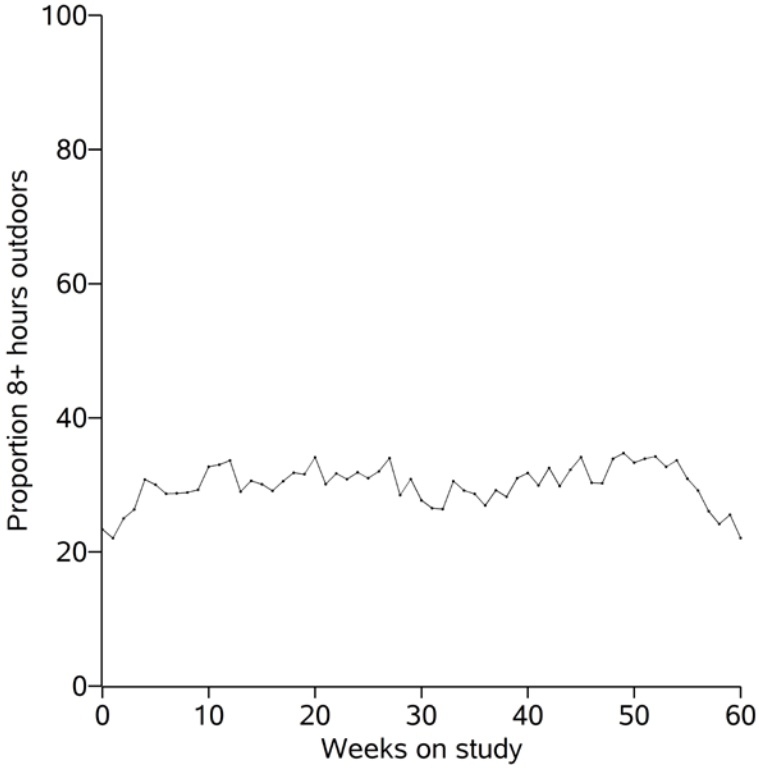


Figure A.3.3 UV index value for participants by time on study. Line follows the median UV index value.

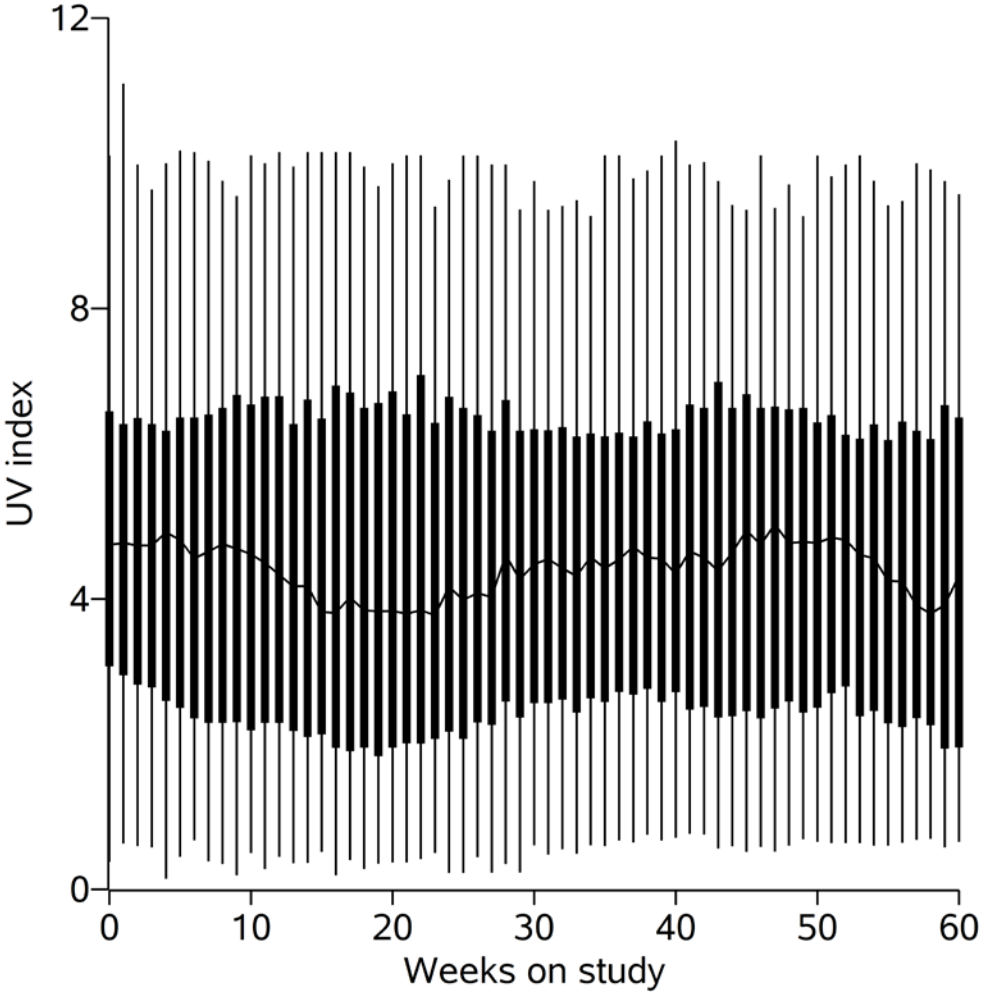


Figure A.3.4 Median stress by weeks on study.

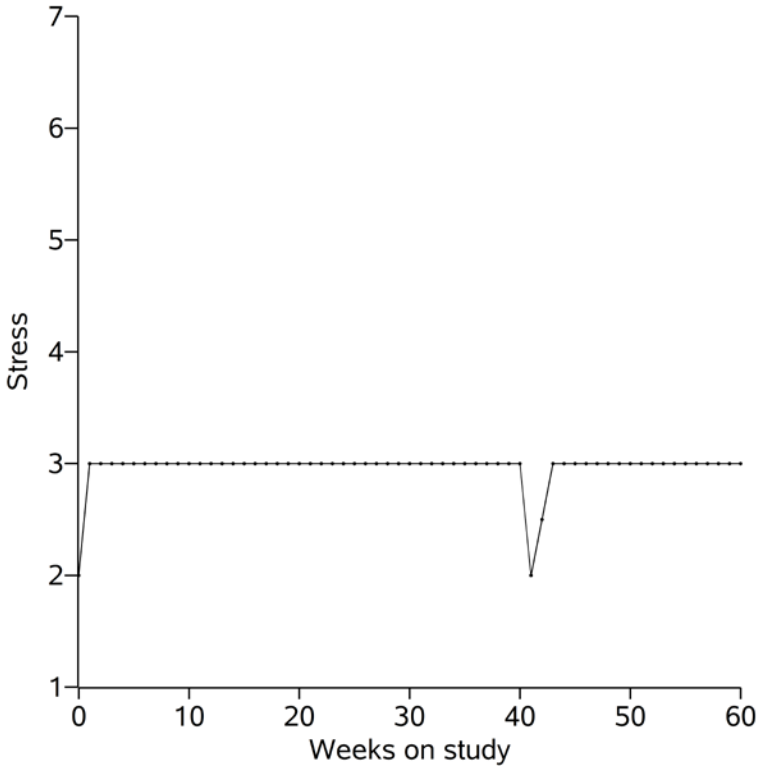


Figure A.3.5 Proportion of participants who use contacts by time on study.

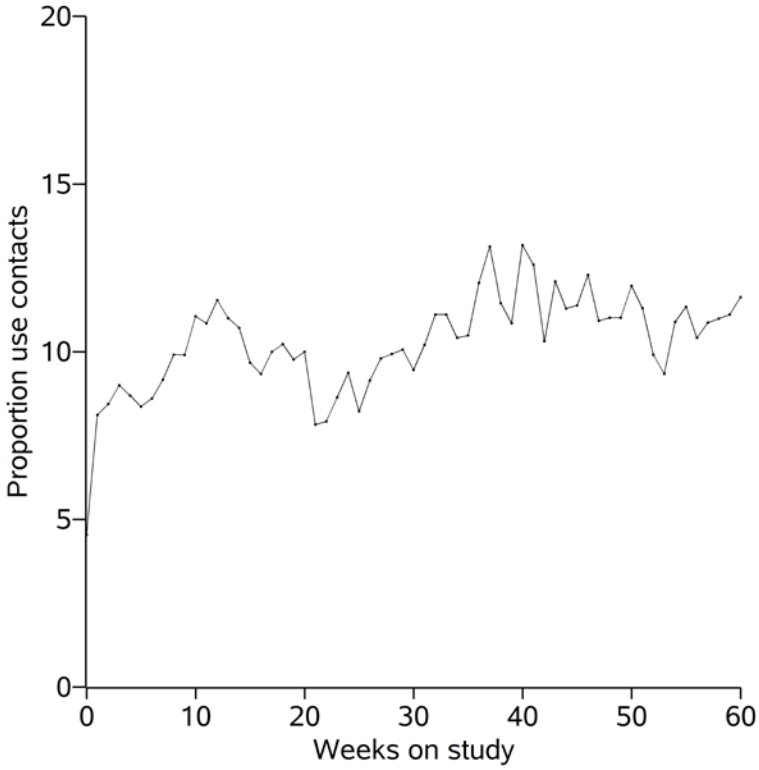


Figure A.3.6 Boxplots of UV index versus reported time spent outdoors pooled over 30 imputations

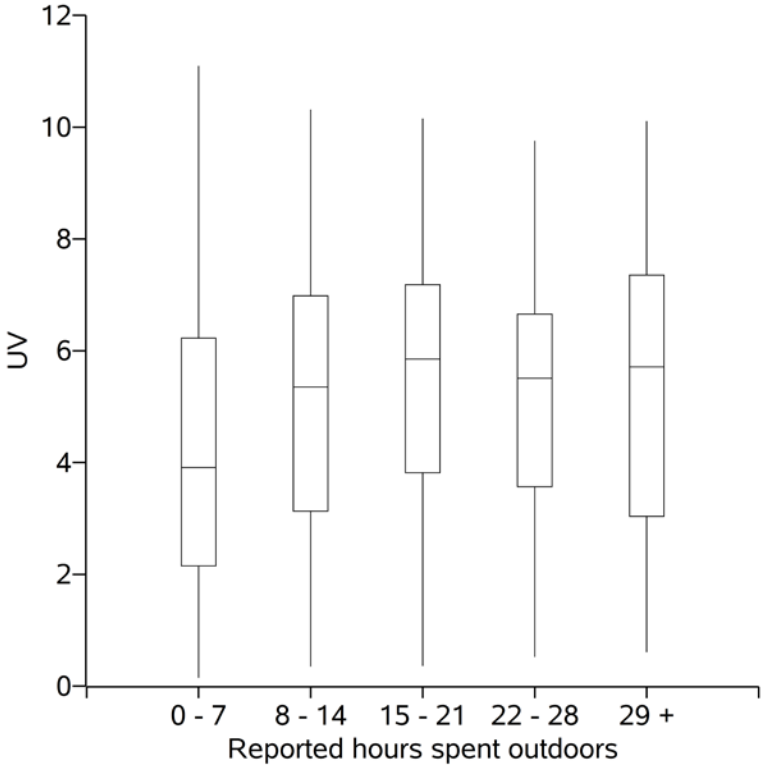
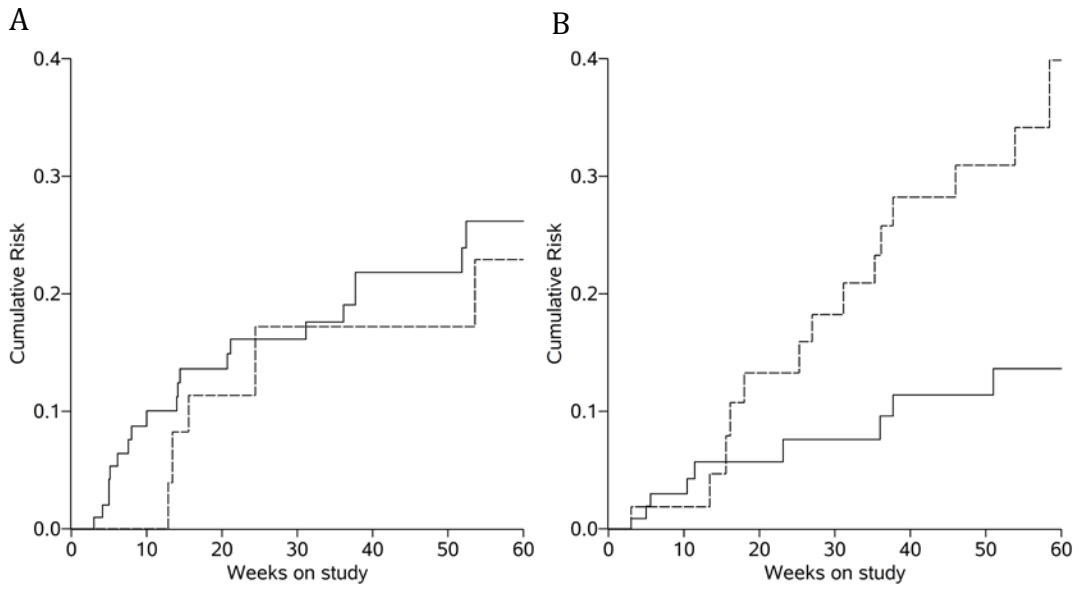


Figure A.3.7 Crude Kaplan-Meier estimates of the cumulative risk of ocular HSV recurrence for person weeks with a UV index value <4 (panel A) and 4+ (panel B) for high sunlight exposure (dashed) and low sunlight exposure (solid) pooled over 30 imputations in 308 Herpetic Eye Disease Study participants.



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