Prevalence and Clinical Consequences of Herpes Simplex Virus Type 1 DNA in Human Cornea Tissues

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(See the editorial commentary by Hill and Clement, on pages 1–4.)

Background. We determined the prevalence and clinical consequences of herpes simplex virus (HSV) type 1 (HSV-1), HSV type 2 (HSV-2), and varicella-zoster virus (VZV) in cornea tissues obtained after penetrating keratoplasty (PKP) was performed.

Methods. The excised corneas of 83 patients with a history of herpetic keratitis (HK; hereafter referred to as “patients with HK”) and 367 patients without a history of HK (hereafter referred to “patients without HK”) were analyzed by real-time polymerase chain reaction (PCR) and virus culture for the presence of HSV-1, HSV-2, and VZV. In addition, 273 post-PKP donor corneoscleral rims were analyzed. The medical records of the transplant patients were reviewed to determine the risk factors influencing intracorneal viral load and graft survival.

Results. HSV-1 was the most prevalent herpesvirus. Both the prevalence of HSV-1 and the HSV-1 DNA load were higher in the corneas of patients with HK than in those of patients without HK. The HSV-1 DNA load in the corneas of patients with HK correlated with age, the recurrence-free interval, cornea neovascularization, steroid treatment before PKP, and disease severity. Herpesvirus DNA was detected in 2 of 273 corneoscleral rims. Graft survival was inversely correlated with the corneal HSV-1 DNA load in patients with HK.

Conclusions. The data presented in this study argue for the implementation of real-time HSV-1 PCR to analyze the excised corneas of patients with HK, to improve post-PKP diagnosis and therapy. Screening of donor corneal tissues for herpesviruses is redundant to prevent newly acquired post-PKP HK.
patients who receive transplants for reasons unrelated to ocular herpesvirus infections [14–16]. This clinical entity, defined as newly acquired HK, occurs in ~9% of all PKP patients [14]. Recurrent HK is predominantly initiated by reactivation of the endogenous latent HSV-1 strain [17]. Alternatively, the virus may be acquired from donor corneal tissue (i.e., via graft-to-host transmission) [18–21].

Because the visual prognosis of patients with post-PKP HK is poor, the identification of PKP patients who are at risk is of major importance [10–13]. Detection and quantification of α-HHV DNA in recipient and donor corneal tissues obtained at the time of PKP may be of diagnostic value in identifying patients who are at high risk for the development of post-PKP HK. To our knowledge, this issue has not been examined extensively [22–25].

The aim of this prospective, cross-sectional study was 2-fold. First, we determined the prevalence and quantity of HSV-1, HSV type 2 (HSV-2), and VZV DNA in the excised corneas from a large cohort of 83 patients with a clinical history of HK who were undergoing PKP (hereafter referred to as “patients with HK”) and 367 patients without a clinical history of HK (table 1). The classification of HK was defined on the basis of clinical criteria [3, 4]. A total of 83 patients with HK and 367 patients without HK (table 1). The classification of HK was defined on the basis of clinical criteria [3, 4]. A total of 83 patients with HK and 367 patients without HK (table 1).

### Table 1. Characteristics of cornea transplant patients and the no. of excised corneal buttons that contain human α-herpesvirus DNA.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Subjects, no. (%)</th>
<th>Sex, ratio of females to males</th>
<th>Subject age, mean ± SD, years</th>
<th>Corneas with viral DNA, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HSV-1 HSV-2 VZV</td>
</tr>
<tr>
<td>Patients with HK (n = 83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious epithelial keratitis</td>
<td>1 (1)</td>
<td>0:1</td>
<td>36</td>
<td>0 0 0</td>
</tr>
<tr>
<td>ISK</td>
<td>22 (27)</td>
<td>13:9</td>
<td>64 ± 16</td>
<td>8 (36) 0 0</td>
</tr>
<tr>
<td>NSK</td>
<td>35 (42)</td>
<td>12:23</td>
<td>56 ± 16</td>
<td>22 (63) 0 1 (3)</td>
</tr>
<tr>
<td>Herpetic keratouveitis</td>
<td>2 (2)</td>
<td>1:1</td>
<td>43 ± 13</td>
<td>1 (50) 0 0</td>
</tr>
<tr>
<td>Endotheliitis</td>
<td>3 (4)</td>
<td>1:2</td>
<td>56 ± 26</td>
<td>0 0 0</td>
</tr>
<tr>
<td>AF with initial HSK diagnosis</td>
<td>20 (24)</td>
<td>6:14</td>
<td>65 ± 13</td>
<td>9 (45) 0 0</td>
</tr>
<tr>
<td>Patients without HK (n = 367)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>53 (14)</td>
<td>24:28</td>
<td>63 ± 19</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Corneal decompensation</td>
<td>9 (2)</td>
<td>2:7</td>
<td>56 ± 17</td>
<td>2 (22) 0 0</td>
</tr>
<tr>
<td>Bullous keratopathy</td>
<td>23 (6)</td>
<td>16:7</td>
<td>66 ± 19</td>
<td>4 (17) 0 2 (9)</td>
</tr>
<tr>
<td>Pseudophake bullous keratopathy</td>
<td>50 (14)</td>
<td>23:27</td>
<td>73 ± 9</td>
<td>7 (14) 2 (4) 0</td>
</tr>
<tr>
<td>Fuchs endothelial dystrophy</td>
<td>97 (26)</td>
<td>62:35</td>
<td>72 ± 10</td>
<td>0 2 (1) 0</td>
</tr>
<tr>
<td>Keratoconus</td>
<td>57 (16)</td>
<td>18:39</td>
<td>36 ± 13</td>
<td>2 (4) 1 (2) 1 (2)</td>
</tr>
<tr>
<td>Trauma</td>
<td>21 (6)</td>
<td>7:14</td>
<td>43 ± 20</td>
<td>0 1 (5) 0</td>
</tr>
<tr>
<td>Other</td>
<td>57 (16)</td>
<td>30:27</td>
<td>55 ± 22</td>
<td>0 1 (2) 2 (4)</td>
</tr>
</tbody>
</table>

**NOTE.** AF, allograft failure; HK, herpetic keratitis; HSK, herpetic stromal keratitis; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; ISK, immune stromal keratitis; NSK, necrotizing stromal keratitis; VZV, varicella-zoster virus.

a At the time of corneal transplantation.

b Patients with a history of HK.

c Patients without a history of HK.

d For example, scrofulosisis and macular corneal dystrophy.

the recipient corneal buttons, as well as the surplus corneoscleral rims of donor corneas obtained after PKP, might influence graft survival.

### MATERIALS AND METHODS

**Patients and clinical specimens.** Corneal buttons were obtained from 450 consecutively seen patients who, during the period from 1999 through 2004, were undergoing therapeutic PKP for visually disabling corneal disease at the Rotterdam Eye Hospital (Rotterdam, The Netherlands). The cohort consisted of 83 patients with HK and 367 patients without HK (table 1). The classification of HK was defined on the basis of clinical criteria [3, 4]. A total of 450 recipient corneal buttons, 273 surplus corneoscleral rims of donor corneas obtained after trepanation of the central cornea, and 84 clear eye bank corneas rejected for transplantation purposes because of low endothelial cell counts (<2300 cells/mm²) were stored at −70°C by 4 h after surgery.

The records of the patients were reviewed to determine the clinical characteristics of the patients before PKP as well as during and at the end of follow-up (~4 years after the procedure was performed). The clinical entities that were scored were age, sex, regimen, and response to preoperative therapy (steroids or an-
viral), recurrence-free interval (RFI), cornea vascularization, post-PKP recurrences of HK, cornea epithelial defects or secondary infections, and graft status at the end of follow-up. A graft failure event was scored when there was partial loss of cornea transparency or complete graft failure. In the group of patients with HK, 62 and 21 patients underwent a primary PKP or a cornea regraft, respectively. Cornea vascularization was scored by counting quadrants of deep vascularization. In contrast to patients without HK, 50 patients with HK (60%) received pre-PKP topical steroid treatment that consisted of fluorometholone-acetate (0.1%) (12 patients), dexamethasone-di-natrium-phosphate (0.1%) (29 patients), or prednisolone-acetate (1%) (9 patients). Long-term pre-PKP systemic acyclovir prophylaxis was given to 39 patients with HK (47%). The RFI, defined as the time from the last episode of clinical HK until the time that PKP was performed, varied from 0.5 to 468 months (mean RFI [± standard deviation [SD]], 39.6 ± 78.4 months). The study was performed according to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Written, informed consent was obtained.

Nucleic acid extraction and complementary DNA (cDNA) synthesis. The cornea tissues were divided through the center of the cornea into 2 equal parts. Tissues were triturated and DNA was isolated using the MagnaPure DNA Tissue Kit II (Roche Diagnostics) combined with the MagnaPure LC Isolation Station (Roche Diagnostics). The eluted DNA was resuspended in 50 μL of elution buffer. Total RNA was isolated from triturated 25% surplus corneal button tissues that contained >1 HSV-1 genome-equivalent copies (gec) per corneal cell, by use of Trizol (Invitrogen). The RNA was reversed transcribed using random hexamer primers, deoxynucleoside triphosphates, and reverse transcriptase (Superscript II), in accordance with the instructions of the manufacturer (Invitrogen). The isolation of DNA and RNA was performed as described elsewhere [26].

Detection of α-HHV DNA by polymerase chain reaction (PCR). The α-HHV genomes were detected by 2 different PCR assays: conventional PCR and real-time quantitative PCR (qPCR) assays. The conventional qualitative PCR assays included amplification of the isolated DNA with α-HHV–specific primers and subsequent Southern blot analysis performed as described elsewhere [27]. The qPCR assays were performed as individual assays in which the human single-copy housekeeping gene Homo sapiens hydroxymethylbilane synthase (HMBS) was run in parallel with each virus target sequence of interest [28]. The sequences and target genes of the primers/probe pairs that were used have been published elsewhere: HSV-1 genes US4 (glycoprotein G) [29], latency-associated transcript (LAT) [30], UL44 (glycoprotein C [gC]) [31], and UL54 (infectious cell protein 27 [ICP27]) [31]; HSV-2 gene US6 (glycoprotein D) [29]; VZV gene 38 [29]; and the HMBS gene [28]. Amplification of the DNA and cDNA samples and detection were performed using an ABI Prism 7700 sequence detection system (Applied Biosystems), as described elsewhere [29]. For standardization of quantitative virus-detection assays, commercially available quantified DNA control panels (Advanced Biotechnologies) and high-titer virus preparations derived from culture supernatants were used [26, 29]. The sensitivity of the qPCR assays, as defined by the 95% hit rate on the electron microscopy–counted virus stocks, was ~200 α-HHV gec/mL.

Virus culture. Human embryonic lung fibroblasts were grown in 24-well plates in medium consisting of Dulbecco’s modified essential medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (Invitrogen). For each assay, a 25% surplus corneal button tissue section from selected case patients was triturated in phosphate-buffered saline, inoculated on the monolayers, and evaluated for cytopathic effect regularly for 2 weeks. When a cytopathic effect was observed, the viruses were typed by immunofluorescence [29].

Statistical analyses. Clinical and laboratory data were analyzed using the SPSS statistical software package (version 15; SPSS). Spearman’s rank correlation test was used to detect associations between viral load and the age, RFI, and graft survival of the recipients. Fisher’s exact test was used to compare categorical data. The Mann-Whitney U test or the Kruskal-Wallis test was used to compare the means of 2 or multiple groups, respectively. Graft survival was calculated using the Kaplan-Meier method and was compared between groups by use of the log-rank test.

RESULTS

Prevalence of HSV-1, HSV-2, and VZV in recipient and donor cornea tissues. To determine the prevalence of α-HHV in human cornea tissues, the excised corneas of patients undergoing PKP and the corneociliary rims from donors were prospectively collected during the period from 1999 through 2004. The patients were divided into 2 groups, on the basis of clinical findings: patients with HK (n = 83) and patients without HK (n = 367) (table 1).

The prevalence of α-HHV DNA in corneas was determined by conventional qualitative PCR (for 22 patients with HK and 53 patients without HK) or qPCR (for 61 patients with HK and 314 patients without HK). The reason why the separate assays were used was that the qPCR assay was established and validated for research purposes at our laboratory in early 2003. Unfortunately, samples assayed by qualitative PCR could not be reanalyzed by qPCR. The data from both assays were combined when appropriate. HSV-1 DNA was detected more frequently in the corneas of patients with HK than in the corneas of patients without HK (40 [48%] of 83 patients and 15 [4%] of 367 patients, respectively) (P < .001, Fisher’s exact test) (table 1). Moreover, the median HSV-1 DNA load (± interquartile
range (IQR) was significantly higher in the corneas of patients with HK than in those of patients without HK (0.20 ± 5.1 HSV-1 gec/cornea cell [range, 0.0003–1548 HSV-1 gec/cornea cell] and 0.002 ± 0.11 HSV-1 gec/cornea cell [range, 0.0004–500 HSV-1 gec/cornea cell], respectively) (P = .012) (figure 1A). In comparison, HSV-2 and VZV were detected more frequently in the corneas of patients without HK than in the corneas of patients with HK (table 1). The median HSV-2 and VZV DNA load (± IQR) in the corneas of patients without HK was 0.0007 ± 0.02 HSV-2 gec/cornea cell (range, 0.0002–2.69 HSV-2 gec/cornea cell) and 0.007 ± 0.02 VZV gec/cornea cell (range, 0.0004–0.264 VZV gec/cornea cell), respectively. No cornea contained DNA of multiple α-HHV (data not shown).

Several corneas of patients with HK (n = 10) and patients without HK (n = 2) who did not show signs of active disease at the time of surgery had relatively high HSV-1 DNA loads (>1 HSV-1 gec/cornea cell), suggesting virus replication in the excised corneas. This issue was investigated by determining the expression of selected HSV-1 transcripts and culturing the virus from the surplus cornea tissue on indicator cells (table 2). During a lytic infection, the HSV-1 genes are sequentially expressed in infected cells in a temporal cascade: immediate early

Figure 1. Correlations between clinical entities and herpes simplex virus type 1 (HSV-1) DNA load in the excised corneas of cornea transplant patients. A, Intracorneal HSV-1 DNA load in transplant patients with a history of herpetic keratitis (HK, hereafter referred to “patients with HK”) and in those without a history of HK (hereafter referred to “patients without HK”). B and C, Intracorneal HSV-1 DNA loads in patients with HK (B) and patients without HK (C) in relation to the age of the patient. D, Combinatory human α-herpesvirus DNA load in the corneas of patients without HK in relation to the age of the patients. Data in panel D are intracorneal HSV-1 (black symbols), herpes simplex virus type 2 (HSV-2) (white symbols), and varicella-zoster virus (VZV) (gray symbols) DNA loads in relation to the age of patients without HK. E–H, Intracorneal HSV-1 DNA load in patients with HK in relation to preoperative treatment with steroids (E) or antivirals (F), corneal vascularization (G), and disease entity (H). The presence of HSV-1 DNA (G) and the HSV-1 DNA load (H) in the corneas are shown in relation to the recurrence-free interval. Conventional box-and-whisker plots are shown in panels A and E–I, and conventional scatter plots are shown in panels B–D and J. The statistical analyses used were the Mann-Whitney U test (A, E, F, and I), the Kruskal-Wallis test (G and H), and Spearman’s rank correlation test (B–D and J). The nos. in the bar graphs denote the no. of patients in each subgroup. ISK, immune stromal keratitis; negative, HSV-1 DNA–negative corneas; NSK, necrotizing stromal keratitis; positive, HSV-1 DNA–positive corneas; rePKP, corneal regraft.
HSV-1 DNA was detected in only 2 (1%) of 273 donor corneoscleral rims obtained after PKP and in 84 clear eye bank corneas rejected for PKP purposes. Although none of the eye bank corneas contained HSV-1 DNA, the HSV-1 DNA load correlated significantly with the age of the patients (figure 1B [r = 0.491; P = .009] and 1C [r = 0.582; P = .029], respectively). In the corneas of patients without HK, this correlation was almost significant when the viral loads of all α-HHV were combined (figure 1D) (r = 0.370; P = .058). Preoperative treatment with antivirals or steroids influenced the HSV-1 DNA load in the corneas of patients with HK. The viral load was significantly higher in patients treated with steroids than in patients treated with antivirals (figure 1E) (P = .002), whereas antiviral treatment tended to correlate with higher corneal HSV-1 DNA loads (figure 1F) (P = .16). The latter observation is most likely the result of the use of antiviral treatment for patients at our hospital who have more severe cases of HK. The types of antivirals and steroids used preoperatively did not correlate with the intracorneal HSV-1 DNA load (data not shown). The HSV-1 DNA load in the corneas of patients with HK diminished gradually with an increasing grade of cornea vascularization (figure 1G) (P = .20).

### Table 2. Detection of herpes simplex virus type 1 (HSV-1) transcripts and infectious virus in HSV-1 DNA-positive corneal explant buttons.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Clinical diagnosis</th>
<th>HSV-1 gec/cell</th>
<th>RFI, months</th>
<th>Relative HSV-1 transcript</th>
<th>HSV-1 culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>73</td>
<td>M</td>
<td>NSK</td>
<td>466</td>
<td>6</td>
<td>61 639</td>
<td>9391 9151</td>
</tr>
<tr>
<td>108</td>
<td>75</td>
<td>M</td>
<td>rePKP for HK</td>
<td>1548</td>
<td>8</td>
<td>163 377</td>
<td>2977 2664</td>
</tr>
<tr>
<td>161</td>
<td>52</td>
<td>M</td>
<td>rePKP for HK</td>
<td>26</td>
<td>6</td>
<td>212 177</td>
<td>2091 2583</td>
</tr>
<tr>
<td>209</td>
<td>72</td>
<td>M</td>
<td>NSK</td>
<td>16</td>
<td>1</td>
<td>1 32</td>
<td>539 832</td>
</tr>
<tr>
<td>217</td>
<td>84</td>
<td>F</td>
<td>FED</td>
<td>500</td>
<td>NA</td>
<td>62 294</td>
<td>586 1006</td>
</tr>
<tr>
<td>249</td>
<td>76</td>
<td>F</td>
<td>rePKP for HK</td>
<td>19</td>
<td>6</td>
<td>3 6</td>
<td>70 139</td>
</tr>
</tbody>
</table>

**NOTE.** FED, Fuchs endothelial dystrophy; gC, glycoprotein C; gG, glycoprotein G; gec, genome-equivalent copies; HK, herpetic keratitis; ICP27, infectious cell protein 27; LAT, latency-associated transcript; NA, not applicable; NSK, necrotizing stromal keratitis; rePKP, corneal regraft; RePKP for HK, regraft for recurrent HK in a graft; RFI, recurrence-free interval.

a At the time of surgery.

b No. of HSV-1 genome-equivalent copies per corneal cell.

c Time in months from the last clinical episode of HK until cornea transplantation.

d Mean relative transcript levels of the indicated HSV-1 genes normalized for β-actin.

e Recovery of infectious virus from the affected corneal tissue.

### Table 3. Prevalence of human α-herpesvirus DNA in recipient and donor cornea tissues.

<table>
<thead>
<tr>
<th>Origin of cornea tissue</th>
<th>HSV-1</th>
<th>HSV-2</th>
<th>VZV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excised cornea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Of patients with HKa (n = 83)</td>
<td>40 (48)</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Of patients without HKb (n = 367)</td>
<td>15 (4)</td>
<td>7 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><strong>Corneoscleral rim (n = 273)</strong></td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Eye bank cornea (n = 84)</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** HK, herpetic keratitis; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VZV, varicella-zoster virus.

a Patients with a history of HK.

b Patients without a history of HK.
higher numbers of patients with HK who had HSV-1 DNA–positive corneas had fulminant disease (data not shown), and patients with HK who underwent regrafting for HK had significantly higher corneal HSV-1 loads than did patients with immune stromal keratitis (figure 1H) \( (P = .041) \). Interestingly, the RFI was significantly shorter among patients with HK who had HSV-1 DNA–positive corneas than among those who had HSV-1 DNA–negative corneas (figure 1I) \( (P = .008) \). The inverse correlation between the RFI and the corneal HSV-1 DNA load affirms this observation (figure 1J) \( (r = -0.385; P = .047) \).

**Correlation between the intracorneal HSV-1 DNA load and graft survival.** At the end of follow-up (mean duration \[ ± SD \], 52 ± 30 months), 27 (33%) of 83 patients with HK who received transplants and 107 (29%) of 367 patients without HK who received transplants experienced graft failure. In both patient groups, graft failure was more common among patients with a history of previous graft rejection in the operated eye. Cornea regraft failure occurred at a similar rate between patients with HK and those without HK: 10 (48%) of 21 and 31 (54%) of 57 cornea regrafts were rejected, respectively.

Next, the PCR data were linked to the clinical records of the patients to determine whether intracorneal HSV-1 DNA may have affected graft survival. Patients with HK who had HSV-1 DNA–positive corneas tended to have a higher risk of graft failure (figure 2A) \( (P = .057) \). The significant inverse correlation between the intracorneal HSV-1 DNA load and graft survival in patients with HK (figure 2B) \( (r = -0.75; P = .026) \) strengthens this association. In contrast, the presence of HSV-1 DNA in the corneal buttons of patients without HK did not significantly affect graft survival (figure 2C) \( (P = .84) \). The hazard ratio (HR) was 0.90 with a 95% confidence interval (CI) of 0.35–2.35. The numbers of VZV DNA–positive and HSV-2 DNA–positive corneal buttons in patients without HK did not significantly affect graft survival (figure 2B) \( (r = -0.075; P = .84) \) and patients without HK who received transplants experienced graft failure. In both patient groups, graft failure was more common among patients with a history of previous graft rejection in the operated eye. Cornea regraft failure occurred at a similar rate between patients with HK and those without HK: 10 (48%) of 21 and 31 (54%) of 57 cornea regrafts were rejected, respectively.

Of the other clinical factors potentially influencing graft survival, only cornea vascularization and preoperative treatment with steroids were significantly different. Preoperative steroid treatment of patients with HK significantly reduced graft survival (figure 2C) \( (P = .016) \) (HR, 2.99 [95% CI, 1.20–5.87]). Both in patients with HK and in patients without HK, graft survival was lower in corneas with ≥1 quadrant of deep cornea vascularization (HR, 2.14 [95% CI, 1.04–5.82]) \( [P = .042] \) [figure 2E] and 2.10 [95% CI, 1.54–4.34] \( [P = .003] \) [figure 2F], respectively). The presence of HSV-1 DNA in corneal buttons did not correlate with the number of post-PKP recurrences of HK, corneal epithelial defects, or secondary infections (data not shown).

Next, we addressed the possibility of identifying patients undergoing PKP who are at risk of acquiring HSV-1 from the corneal graft. As described above, only 2 of 273 corneoscleral corneal grafts were too small to be analyzed. Of the other clinical factors potentially influencing graft survival, only cornea vascularization and preoperative treatment with steroids were significantly different. Preoperative steroid treatment of patients with HK significantly reduced graft survival (figure 2C) \( (P = .016) \) (HR, 2.99 [95% CI, 1.20–5.87]). Both in patients with HK and in patients without HK, graft survival was lower in corneas with ≥1 quadrant of deep cornea vascularization (HR, 2.14 [95% CI, 1.04–5.82]) \( [P = .042] \) [figure 2E] and 2.10 [95% CI, 1.54–4.34] \( [P = .003] \) [figure 2F], respectively). The presence of HSV-1 DNA in corneal buttons did not correlate with the number of post-PKP recurrences of HK, corneal epithelial defects, or secondary infections (data not shown).

Next, we addressed the possibility of identifying patients undergoing PKP who are at risk of acquiring HSV-1 from the corneal graft. As described above, only 2 of 273 corneoscleral rims contained HSV-1 DNA, and HSV-2 DNA and VZV DNA could not be detected. Both HSV-1 DNA–positive grafts were transplanted into patients with HK who, at the end of follow-up in May 2008, did not develop post-PKP HK or graft failure. Furthermore, none of the patients without HK who received α-HHV DNA–positive cornea transplants developed HK, and 6 patients without HK developed culture-proven, newly ac-
quired HK. However, none of these patients had α-HHV DNA in their corneas or received a graft in which α-HHV DNA was detectable in the corneoscleral rim of the donor (data not shown). Because all 6 patients without HK were HSV-1 seropositive, the newly acquired HK was most likely caused by reactivation of the endogenous HSV-1 strain.

DISCUSSION

In the present study, we determined the prevalence and diagnostic value of detecting α-HHV–specific DNA in human donor and recipient cornea tissues. Three main findings are reported. First, α-HHV DNA was rarely detected in donor cornea tissues and in the excised corneas of patients without HK. Second, the viral DNA load of HSV-1, the most prevalent α-HHV detected in the corneal buttons analyzed, correlated with the patients’ age in both PKP groups. Third, the HSV-1 DNA load in corneas was inversely correlated with graft survival in patients with HK.

Laboratory diagnostic testing of cornea specimens is of additional value in diagnosing cornea disease. The introduction of qualitative PCR and, more recently, qPCR has greatly improved the sensitivity of detecting the etiologic agent in clinical specimens obtained from persons with suspected cases of infectious keratitis [29]. As reported earlier [22–25], HSV-1 was the most prevalent α-HHV detected in cornea tissues and was more frequently identified in the corneas of patients with HK (40% [48%] of 83 corneal buttons) than in the corneas of patients without HK (15% [4%] of 367 corneal buttons). The low number of HSV-2 DNA– and VZV DNA–positive cornea tissues, as described by other investigators [22–25], is most likely a result of the different anatomical location of latent HSV-2, compared with HSV-1 and VZV, and of the relative low reactivation frequency of VZV, compared with HSV, in immunocompetent individuals [32, 35].

Whereas the data confirm the clinical diagnosis in the majority of patients with HK, detection of α-HHV DNA in the corneas of patients without HK is puzzling. The data obtained for the corneas of patients without HK may reflect cross-contamination or, less likely, misdiagnosis of the cornea disease. A recent study by James Hill and colleagues has shown that infectious virus and, subsequently, HSV-1 DNA become undetectable when immune cells infiltrate the infected cornea [41]. In the present study and in a recent study of a limited number of corneas from patients with HK [25], the corneal HSV-1 DNA load decreased gradually with an increasing RFI (figure 1F). Furthermore, the viral load correlated with fulminant disease (figure 1H) and preoperative steroid treatment (figure 1E), whereas cornea neovascularization was inversely correlated with the corneal HSV-1 DNA load (figure 1G). Compared with clinically quiescent HSV-1 cornea lesions, active HK lesions are densely infiltrated with lymphocytes [2, 41]. Steroids inhibit inflammation, and deep cornea neovascularization facilitates the egress of inflammatory cells into affected tissues [41, 42]. Altogether, the data support the notion that local inflammatory responses are involved in clearing the virus from the infected cornea tissue. This, combined with the detection limit of the PCR assays performed on only one-half of the corneas analyzed, may account for the inability to detect HSV-1 DNA in the remaining one-half of patients with HK who were analyzed (table 1).

An interesting yet not previously reported finding in the present study was that the intracorneal HSV-1 DNA load correlated with the age of the patients in both groups of patients undergoing PKP (figure 1), suggesting that a general phenomenon was involved. Whereas the microbiological spectrum of infectious keratitis is similar between elderly patients (those >60 years old) and younger patients, the incidence of HK and associated morbidity is higher among elderly patients that among younger patients [43, 44]. Similar observations have been made in mice. Unlike young mice, adult mice are more susceptible to severe HSV-1–induced cornea inflammation, which appears to be largely attributable to age-related alterations of the immune system [45]. A decrease in immune function with advancing age is a hallmark of aging. As a result, infectious diseases cause more morbidity and mortality among elderly individuals than among younger individuals [46]. Accordingly, the association between age and the HSV-1 DNA load is most likely due to the age-related deprived immune control of latent HSV-1 in the innervating ganglion [26, 47]. Alternatively, corneas of elderly patients may be more susceptible to HSV-1 infections.

Although the cornea is an immune-privileged site, corneal graft rejection is the major cause of graft failure; this fact argues for the identification and treatment of patients undergoing PKP who are at high risk for graft rejection [1, 2, 6]. Extended cornea neovascularization, a history of graft rejection, and a history of HK in the operated eye are considered to be important high-
risk factors for graft rejection [2, 6]. Analogous to previous reports, corneal neovascularization and cornea regrafting negatively influenced graft survival in both patient groups [1, 2, 6, 7]. In contrast, however, the incidence of graft failure was not significantly different between patients with HK and patients without HK during the 4-year follow-up. This discrepancy may in part be attributable to the fact that our hospital, the only one in The Netherlands that has ophthalmologic diseases as its primary focus, is the national referral center for patients with complicated cornea disease, including severe HK. Alternatively, the favorable results for patients with HK may reflect the instigation of effective combined antiviral and local immunosuppressive therapy immediately after transplantation.

An additional important finding of our study was that the presence of HSV-1 and, in particular, the HSV-1 DNA load in excised corneas negatively influences graft survival in patients with HK but not in patients without HK (figure 2). This parameter correlated with graft survival independently of the aforementioned high-risk factors. However, as would be expected, the corneal HSV-1 load correlated with the severity of HK at the time of surgery. Inevitably, interpretation of the clinical data relies on the experience of the ophthalmologist, arguing for the need to include updated laboratory diagnostic tools to optimize the differential diagnosis without ambiguity.

In conclusion, the data demonstrate that the HSV-1 DNA load in the corneas of patients with HK correlated with age, RFI, cornea neovascularization, pre-PKP steroid treatment, disease severity at the time of surgery, and corneal graft rejection. HSV-1 qPCR assays performed on the excised corneas of patients with HK will improve post-PKP diagnosis and therapy to prevent severe HSV-1–related complications of cornea grafting, especially among elderly patients. In contrast, HSV-1 qPCR assays performed on donor cornea tissues have no diagnostic value in predicting the development of newly acquired HK in patients without HK or graft-to-host transmission of HSV-1 in patients undergoing PKP in general.

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