Multimodal characterization of the features of the course of stromal herpetic keratitis according to clinical, functional, laboratory and morphometric indicators

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Мультимодальна характеристика особливостей перебігу стромального герпетичного кератиту за клінічними, функціональними, лабораторними та морфометричними показниками

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Abstract

Purpose: *To determine the features of the course of herpetic stromal keratitis (HSK) based on a comprehensive evaluation of clinical, functional, laboratory and morphometric parameters.*

Material and Methods. *Totally, 60 patients with HSK (mean age, 39.4 ± 9.5 years) were included in the study. Doppler ultrasound (Toshiba Nemio-20) was used to assess hemodynamics in the ophthalmic artery (OA), central retinal artery (CRA) and central retinal vein (CRV). Pentacam AXL (Oculus, Germany) was used for keratometry, pachymetry and corneal densitometry measurements, and optical computed tomography (Optopol REVO NX, Poland), for corneal epithelial thickness measurements. Corneal neovascularization (NVR) was assessed by the area and depth of vascular invasion; SpO_2 by pulse oxymetry; and immunoglobulin G (IgG) for herpes simplex virus 1 and 2 (HSV 1/2) and cytomegalovirus (CMV), by enzyme-linked immunosorbent assay. Schirmer II and tear film break-up time tests were used to assess tear production and tear film stability.*

Results. *In patients with HSK, the incidence of corneal ulceration increased with the area of corneal NVR ($p = 0.0006$). Peripheral ulceration prevailed in NVR in one quadrant, and central NVR, in ≥2 quadrants ($\chi^2 = 4.3$; $p = 0.04$). The incidence of total corneal edema increased with the area of corneal NVR. Corneal densitometry values were by 20.5% higher in the presence of total edema than in the presence of focal edema. The risk of mixed NVR was significantly increased when ≥2 corneal quadrants were affected by NVR ($p < 0.001$). The incidence of corneal ulceration in superficial, deep and mixed NVR was 25.9%, 16.7% and 100%, respectively. CRA hemodynamics parameters did not depend on the presence of NVR and ulceration, whereas CRV flow rate was 7.2% higher in the presence of NVR ($p < 0.05$). The presence of NVR was associated with a 23.6% reduced tear film stability ($p = 0.004$). We found no association of IgG for HSV 1/2 or CMV with characteristics of corneal NVR and ulceration.*

Conclusion. *Progression of damage to the corneal stroma and development of corneal ulceration were associated with the severity of neovascularization. The incidence of ulceration increased with the number of NVR quadrants (p*

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= 0.0006), and patients with mixed NVR had 4.3 times the odds of ulceration (p < 0.001). NVR depth and keratometry measurements were the most informative for predicting the risk of corneal ulceration. NVR was associated with moderate changes in regional hemodynamics and marked abnormality of tear production with tear film instability.

Keywords: *herpetic keratitis, herpes simplex virus, ocular hemodynamics, tear production, corneal morphometry, cornea*

Резюме

Мета. *Визначити особливості перебігу стромального герпетичного кератиту (СГК) на основі комплексної оцінки клінічних, морфометричних, лабораторних і функціональних показників.*

Матеріал і методи. *Обстежено 60 пацієнтів із монолатеральним СГК, середній вік – 39,4 ± 9,5 р. Гемодинаміку в очній артерії (ОА), центральній артерії (ЦАС) та вені сітківки (ЦВС) досліджували методом доплерографії (Toshiba Nemio-20). Кератометрію, пахіметрію та денситометрію рогівки визначали за допомогою Pentacam AXL (Oculus, Німеччина), товщину епітелію рогівки – методом ОКТ (Optopol REVO NX, Польща). Неоваскуляризацію рогівки (НВР) оцінювали за площею та глибиною інвазії судин, SpO_2 – методом пульсоксиметрії, IgG до ВПГ 1/2 та ЦМВ – методом ІФА. Сльозопродукцію та стабільність слізної плівки оцінювали за тестами Ширмера II та Норна.*

Результати. *У хворих на СГК встановлено зростання частоти виразки рогівки зі збільшенням площі НВР (p = 0,0006). Периферична виразка переважала при НВР одного квадранта, тоді як центральна – при ураженні двох і більше квадрантів ($\chi^2 = 4,3$; p = 0,04). Зі збільшенням площі НВР зростала частота тотального набряку рогівки. Денситометрія на 20,5% (p = 0,009) вище при тотальному набряку порівняно з фокальним. При поширенні НВР ≥2 квадрантів значно підвищувався ризик формування змішаної форми НВР (p < 0,001). Частота виразки становила 25,9% при поверхневій, 16,7% – при глибокій та 100% – при змішаній НВР. Показники гемодинаміки ЦАС не залежали від наявності НВР і виразки, тоді як швидкість кровотоку в ЦВС була вищою на 7,2% при НВР (p < 0,05). Наявність НВР асоціювалася зі зниженням стабільності слізної плівки на 23,6% (p = 0,004). Зв'язку між рівнями IgG до ВПГ та ЦМВ і характеристиками НВР та виразкою рогівки не виявлено.*

Висновки. *Прогресування стромального ушкодження рогівки та формування виразкового дефекту пов'язано з вираженістю НВР: зі збільшенням її площі частота виразок зростає (p = 0,0006), а змішаний тип НВР підвищує її ризик у 4,3 раза (p < 0,001). Щодо прогнозування ризику виразки рогівки найбільш інформативними є показники глибини НВР та кератометрії. НВР асоціюється з помірними змінами регіонарної гемодинаміки та вираженим порушенням сльозопродукції із нестабільністю слізної плівки.*

Ключові слова: *герпетичний кератит, вірус простого герпесу, гемодинаміка судин ока, сльозопродукція, морфометрія рогівки, рогівка.*

Introduction

Herpetic keratitis (HK) is a corneal viral infection caused by herpes simplex virus (HSV). The disease is leading cause of infectious blindness in developed countries [1–3]. A 2012 review estimated the global number of HSV keratitis globally to be 1.5 million, including 40,000 new cases of severe monocular visual impairment or blindness each year [4]. In 2016, an estimated 1.7 million people had HSV keratitis [5]. Recurrence rates of ocular HSV after an initial episode have been estimated at 36% at 5 years, and over 60% at 20 years [6]. There are typically four subtypes of HSV keratitis which include: (1) epithelial keratitis; (2) immune stromal keratitis; (3) stromal necrotic keratitis; and (4) endotheliitis [7]. In a study by Kim and colleagues [8], the most frequent subtype of HSV keratitis was epithelial keratitis (49.7%), which was followed by herpetic stromal keratitis (HSK; 23.5%). Although all subtypes of HSV keratitis are typically recurrent, HSK is the most likely subtype to recur. Chronic recurrent stromal keratitis can lead to the development of stromal scarring, corneal thinning and corneal neovascularization (CNV) [9].

Non-necrotizing stromal keratitis is characterized by more diffuse stromal inflammation, whereas necrotizing stromal keratitis is accompanied by marked stromal inflammation and necrosis and is associated with a high risk of corneal ulceration and perforation [2, 10]. Prediction of the development of HSK would be helpful for early identification of patients with a high risk of an unfavorable course (particularly the development of necrotic changes with deep stromal destruction, pathological CNV and corneal scarring). This, in turn, would enable providing a timely, customized treatment strategy taking into account systemic and regional functional parameters and morphological status of the cornea, improving the efficiency of treatment and preventing persistent opacities, astigmatism and irreversible visual function loss.

The purpose of this study was to determine the features of the course of HSK based on a comprehensive evaluation of clinical, functional, laboratory and morphometric parameters.

Material and Methods

Totally, 60 patients (29 men and 31 women; mean age, 39.4 ± 9.5 years) with active unilateral chronic recurrent HSK were examined at the Dnipropetrovsk Regional

Clinical Ophthalmological Hospital. Patients were categorized into two groups based on the classifications by Liesegang [10] and Holland and Schwartz [11]: those with immune HSK (group 1; n = 42) and those with necrotizing HSK (group 2; n = 18).

This study followed ethical standards as outlined in the Declaration of Helsinki of the World Medical Association, ICH guideline for good clinical practice and relevant laws of Ukraine, and was approved by the Bioethics committee of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" (Meeting Minutes No. 2 of April 18, 2025). Informed consent was obtained from all subjects.

A Doppler ultrasound machine (Toshiba Nemio-20, Toshiba Medical Systems, Tokyo, Japan) with a 8-MHz linear transducer, 3-MHz convex transducer, and 3.75-MHz microconvex transducer was used to assess hemodynamics in the ophthalmic artery (OA), central retinal artery (CRA) and central retinal vein (CRV). Peak systolic velocity (PSV; cm/s), end diastolic velocity (EDV; cm/s), and resistance index (Ri) were determined bilaterally in the OA and CRA, and PSV only was determined in the CRV. Fellow eyes were used as controls.

Pentacam AXL (Oculus Optikgeräte GmbH, Wetzlar, Germany) was used for keratometry, pachymetry and corneal optical densitometry (corneal light backscatter measured in grey scale units (GSU)) measurements. Optical computed tomography (Optopol REVO NX, Zawiercie, Poland) for minimum and maximum corneal epithelial thickness measurements and the differences between them.

The numbers of corneal quadrants affected by CNV and invasion depth were evaluated with a slit lamp at maximum illumination and 16x magnification using a narrow slit beam. The vessel depth was calculated as the distance from the anterior corneal surface to the blood vessel. CNV was classified as superficial (with superficial layers only being affected), deep (with stromal layers only being affected) or mixed (with both superficial and stromal layers being affected). Superficial vessels spring from the conjunctival network usually appear bright red and tortuous, while deep vessels from the limbal marginal loop network, dark red and less tortuous [12].

Peripheral capillary oxygen saturation (SpO_2) was assessed by pulse oximetry. A SpO_2 value ranging between 95% and 100% was considered normal.

A tear film break-up time (TBUT) test was used to evaluate tear film stability. A moistened fluorescein strip was applied to the ocular surface without touching the cornea. The patient was asked to blink several times and then look straight forward, without blinking. The tear film was examined at the slit lamp with a broad beam using the cobalt blue filter. After an interval, black spots appeared in the fluorescein-stained film, indicating the formation of dry areas. The time interval between the last blink and appearance of the first dry spot was used for the assessment. A TBUT value of more than 10 s was considered normal, and less than 10 s, abnormal.

Schirmer II test was performed with a topical anesthetic (oxybuprocaine) to assess basal tear production. After the anesthetic was instilled and the conjunctiva was dried, a test strip was placed into the conjunctival sac at the junction of the medial and outer thirds of the lower eyelid without touching the cornea and lashes. The patient was asked to close the eye. The Schirmer's strip was removed after 5 minutes, and the length of the moisture on the strip was measured. A test result of more than 15 mm was considered normal.

An enzyme-linked immunosorbent assay (ELISA) was used to measure immunoglobulin G (IgG) antibody levels to HSV 1/2 and cytomegalovirus (CMV).

The Shapiro-Wilk test or Kolmogorov-Smirnov test was used to determine if the data were normally distributed. The independent t-test was used for group comparison of normally distributed data. Normally distributed data are presented as mean ± standard deviation (SD). Before one-way analysis of variance (ANOVA), a Levene's test of homogeneity of variance ($p > 0.05$) was conducted to allow for use of parametric analysis. The Newman Keuls test was used post hoc for pairwise comparisons if the samples followed a normal distribution and had equal variances. Skewed data are presented as median with interquartile range (IQR). For skewed data, multiple group comparisons were calculated using the Kruskal-Wallis test. Significance levels for differences in frequency among categorical variables were accessed with the chi-square test with the Yates correction. Relative risk (RR) and odds ratio (OR) were used to assess the effect of associations between risk factors and outcomes. RR is defined as the number of events divided by the sample size, whereas the odds are the number of events divided by the number of non-events.

The Haldane-Anscombe correction was applied if any cell count was zero. Cochran-Armitage trend test was applied to assess a linear trend in the change in the percent incidence of ulceration with an increasing number of corneal quadrants affected by CNV across groups. The criterion was used to check for a statistically significant trend to an increase or decrease in the frequency of an event depending on the sequential number of the factor (number of corneal quadrants affected). Test results are presented as Z-scores at $p < 0.05$ [13]. Kullback–Leibler divergence (D_{kl}) measures the difference between two distributions in information units (bits) and was used to assess the diagnostic value. This parameter can be helpful for the quantitative evaluation of the degree of informational difference between groups and objective determination of the diagnostic value of the criterion examined. A D_{kl} smaller than 0.1 bit indicated low diagnostic informativeness, a D_{kl} larger than or equal to 0.1 bit but smaller than 0.5 bit, moderate diagnostic informativeness, and D_{kl} larger than or equal to 0.5 bit, high diagnostic informativeness [14].

Results

The number of corneal quadrants affected by CNV was 0 in 20%, 1 in 50%, 2 in 23.3%, and 3 in 6.7% of study patients. The percent incidence of corneal ulceration was 30% for one corneal quadrant, 35.7% for two corneal quadrants, and 100% for three corneal quadrants affected by CNV (Table 1). The Cochran-Armitage trend test confirmed that there was a statistically significantly increasing trend in the frequency of corneal ulceration with an increase in the number of corneal quadrants affected with CNV ($Z = 3.44$; $p = 0.0006$).

Corneal ulceration was found in 37.5% of patients with HSK associated with CNV, and in no HSK patients without CNV. The difference in the frequency was statistically significant ($\chi^2 = 4.77$; $p = 0.029$). The presence of CNV had a moderate diagnostic power for the presence of corneal ulceration ($D_{kl} \approx 0.6$ bit).

Peripheral corneal ulceration was more common (100%) in cases with only one corneal quadrant affected by CNV ($\chi^2 = 4.3$; $p = 0.04$), and central corneal ulceration (90%), in cases with two or more corneal quadrants affected by CNV. The risk of the presence of central corneal ulceration was 19 times higher in HSK patients with two or more corneal quadrants affected by CNV than in HSK patients without CNV (adjusted RR = 19.0; 95% CI, 1.13–318; $p = 0.003$; Table 1).

Focal corneal edema was seen in 100% of HSK patients with not more than one corneal quadrant affected by CNV, which was twice more common than (as much as 50%) in HSK patients with two or more corneal quadrants affected by CNV ($\chi^2 = 19.3$; $p < 0.001$). This was associated with an increase in the frequency of total corneal edema, the latter being found in 37.5% of HSK patients with two corneal quadrants affected and 100% of HSK patients with three corneal quadrants affected (Table 1). The frequency of corneal edema increased with an increase in the number of corneal quadrants affected by CNV and in the presence of central corneal edema. Corneal densitometry was 34.5 ± 12.7 GSU in HSK patients with total corneal edema, and was 20.5% lower (27.4 ± 7.1 GSU; $p = 0.009$) in HSK patients with focal corneal edema.

In the study sample of patients with HSK, superficial CNV was most common (56.25%), followed by deep CNV (25%) and mixed CNV (18.75%) ($\chi^2 = 11.625$; $df = 2$; $p = 0.003$) (Table 2). The majority (92.6%) of patients with superficial CNV had only one corneal quadrant affected by CNV. Of the HSK patients with deep CNV, 41.7% had one corneal quadrant affected, and 58.3%, two corneal quadrants affected. Mixed CNV was seen only in HSK patients with ≥ 2 corneal quadrants affected. Superficial CNV was extremely rare in patients with a large area of neovascularization (≥ 2 quadrants affected). Patients with ≥ 2 quadrants affected, however, had 19 times the odds of having mixed CNV (OR = 63; 95% CI, 3.36–1190; $p < 0.001$; Table 2).

There was a significant difference in the frequency of corneal ulceration among the groups of HSK patients with superficial, deep and mixed CNV ($\chi^2 = 11.9$ $df = 2$; $p = 0.003$) (Table 2). That is, the depth of CNV was a significant factor for the presence of corneal ulceration. Corneal ulceration was seen in 25.9%, 16.7% and 100% of HSK patients with superficial, deep and mixed CNV, respectively. That is, in patients with mixed CNV, corneal ulceration was four times more common than in patients with superficial CNV ($\chi^2 = 15$, $p < 0.001$) and six times more common than in patients with deep CNV ($\chi^2 = 14.3$, $p < 0.001$), and these differences were significant.

The probability of developing ulceration was 4.3 times higher in patients with mixed CNV than in those with superficial or deep CNV (RR = 4.3; 95%CI, 2.25–7.09), $p < 0.001$). The depth of CNV had a diagnostic value for the presence of corneal ulceration ($D_{kl} \approx 0.9$ bit, indicating a high informativeness of this factor).

Corneal epithelial thickness in HSK patients with and without CNV and ulceration, and between those with different numbers of quadrants or depth affected by CNV.

Table 1. Incidence of clinical features of HSK in groups with no, one, two or three corneal quadrants affected by corneal neovascularization

Feature	No CNV		One quadrant affected by CNV		Two quadrants affected by CNV		Three quadrants affected by CNV	
	n=12		n=30		n=14		n=4	
	Feature yes/no (n)							
	yes	no	yes	no	yes	no	yes	no
Total corneal edema	0	12	0	30	5	9	4	0
Focal corneal edema	12	0	30	0	9	5	0	4
Central corneal ulceration	0	12	1	29	5	9	4	0
Peripheral corneal ulceration	0	12	8	0	0	0	0	0

Note: CNV, corneal neovascularization; HSK, herpetic stromal keratitis; n, number of eyes

Table 2. Incidence of superficial, deep and mixed corneal neovascularization in HSK groups with one, two or three corneal quadrants affected by corneal neovascularization in the presence or absence of corneal ulceration

Superficial, mixed or deep corneal neovascularization	Number of eyes						Total
	One quadrant affected by CNV (n=30)		Two quadrants affected by CNV (n=14)		Three quadrants affected by CNV (n=4)		eyes n = 48
	No ulceration	Corneal ulceration	No ulceration	Corneal ulceration	No ulceration	Corneal ulceration	
Superficial	18	7	2	0	0	0	27
Deep	3	2	7	0	0	0	12
Mixed	0	0	0	5	0	4	9

Note: CNV, corneal neovascularization; HSK, herpetic stromal keratitis; n, number of eyes

Table 3. Mean ± standard deviation of hemodynamics parameters of the central retinal artery and central retinal vein in HSK groups with no, one, two or three corneal quadrants affected by corneal neovascularization

Hemody-namics parameter	No CNV	One quadrant affected by CNV	Two quadrants affected by CNV	Three quadrants affected by CNV	P-value
	n = 12	n = 30	n = 14	n = 4	
	1	2	3	4	
CRA PSV	18.7 ± 1.7	17.9 ± 2.4	17.9 ± 3.8	19.1 ± 3.8	–
CRA ESV	5.37 ± 0.61	5.76 ± 1.4	6.21 ± 1.62	5.51 ± 0.77	–
CRA IR	0.67 ± 0.02	0.68 ± 0.02	0.68 ± 0.02	0.68 ± 0.04	–
CRV PSV	6.84 ± 0.25	7.27 ± 0.47	7.4 ± 0.98	7.42 ± 0.59	P_{1-2} = 0.006 P_{1-3} = 0.02 P_{1-4} = 0.005

Note: CNV, corneal neovascularization; CRA, central retinal artery; CRV, central retinal vein; EDV, end-diastolic velocity; HSK, herpetic stromal keratitis; IR, index of resistance; n, number of eyes; PSV, peak systemic velocity in the ophthalmic artery;

Maximum and minimum corneal epithelial thickness were 69.5 ± 8.5 μm and 51.5 ± 5.0 μm, respectively, for patients without corneal ulceration, 71.3 ± 10.2 μm and 50.6 ± 5.0 μm, respectively, for patients with central corneal ulceration, and 71.4 ± 6.9 μm and 50.6 ± 5.0 μm, respectively, for patients with peripheral corneal ulceration, with no significant between group difference (the sector affected by ulceration was excluded from the calculation). The difference between maximum and minimum corneal epithelial thickness reflects the degree of unevenness of corneal epithelial thickness, and tended to increase in patients with ulceration (20.1 ± 5.2 μm), with no significant between group difference (p = 0.07). An ANOVA showed no significant association between the depth of CNV and the difference between maximum and minimum corneal epithelial thickness (F(3.56) = 1.12; p = 0.35), which was confirmed by the Newman Keuls test. Mild variability in the difference between maximum and minimum corneal epithelial thickness among the groups indicates its low discriminative power and limited informativeness, likely, in the presence of corneal edema.

Corneal curvature as assessed by keratometry in HSK patients with versus without CNV. Mean keratometry reading was significantly lower in HSK patients without ulceration than in those with ulceration (41.2 ± 3.2 D versus 42.5 ± 1.8 D; p = 0.03). A D_{kl} of 0.11 bit indicates low diagnostic informativeness of the parameter: it discriminates the groups of patients partially and may be used as an additional differentiation criterion but is not a completely discriminating factor. We found no association of the keratometry reading with the depth of CNV (F =0.88, p = 0.46).

Pachymetry readings depending on the presence of CNV and ulceration in HSK patients. No significant between group difference in the presence of CNV and ulceration in patients with HSK was observed, likely due to a complex effect of inflammatory edema, stromal destruction and reparative processes. Mean pachymetry reading was 551.5 μm (95% CI, 536–566 μm).

Ophthalmic hemodynamics in HSK patients without versus with CNV and those with different numbers of quadrants affected by CNV. There was a significant difference in the blood flow velocity in the CRV, but not in hemodynamic parameters (PSV, EDV and IR) in the CRA between groups with versus without CNV (Table 3). The PSV in the CRV was significantly lower in the group without CNV than in the groups with one, two or three affected quadrants, with no significant difference between

the groups with one, two or three affected quadrants. Consequently, further analysis was performed to identify differences in the PSV in the CRV between the group without CNV and the group with CNV. The PSV in the CRV was 7.2% higher in the group with CNV than in the group without CNV (7.33 ± 0.66 cm/s versus 6.84 ± 0.25 cm/s; $p < 0.05$). A D_{kl} for the PSV in the CRV was 0.32 bit, indicating moderate informativeness of the parameter for discriminating the group without CNV from the groups with one, two or three affected quadrants. The PSV in the CRV in HSK patients with and without corneal ulceration was 7.3 ± 0.5 cm/s and 7.2 ± 0.7 cm/s, respectively, but the difference was not significant ($p = 0.2$). That is, the PSV in the CRV had a diagnostic value for CNV, but not for corneal ulceration in HSK patients.

Systemic blood oxygenation in HSK patients. Peripheral capillary oxygen saturation (SpO_2) was characterized by low variability (6.8%), with a mean value of 96.5 ± 0.6 % (95% CI, 96.3–96.6%), which was within the normal range. There was no significant difference in SpO_2 between HSK patients with and without CNV and ulceration, and between those with different numbers of quadrants affected by, or depth of, CNV, which indicated that this characteristic is not informative of the severity of pathological changes in the cornea.

Tear production as assessed by Schirmer II and TBUT tests in HSK patients with and without CNV and ulceration, and between those with different numbers of CNV quadrants. Schirmer II test scores were lower than normal in all groups of HSK patients, with no significant difference between groups ($H = 1.74$; $df = 3$; $p = 0.63$), which indicated no association between the tear production and the area of CNV. The basal tear production (as assessed by Schirmer II test) was higher for HSK patients without than for those with corneal ulceration (6.6 ± 1.8 mm versus 6.2 ± 1.5 mm, respectively), but the difference was not significant ($p = 0.2$) (Table 4). TBUT test scores were by 23.6% lower in HSK patients with one to three CNV quadrants than in those without CNV (2.9 ± 1.0 s versus 3.8 ± 1.1 s, respectively; $p = 0.004$). Kullback–Leibler divergence (D_{kl}) was calculated to compare the distributions in the group without CNV and the group with one to three CNV quadrants. The D_{kl} was approximately 0.77 bit, indicating a moderate difference in the informativeness between these groups. TBUT test scores were higher in HSK patients without than in those with corneal ulceration (3.1 ± 1.0 s versus 2.8 ± 1.0 s, respectively; $p = 0.14$).

Serum levels of HSV- and CMV-specific IgG in HSK patients with and without CNV and ulceration, and between those with different numbers of CNV quadrants. Serum median (IQR) levels of HSV- and CMV-specific IgG in HSK patients were 45.7 (26.9–56.6) and 11.5 (6.5–20.1), respectively. There was no significant difference as assessed by the Kruskall-Wallis H test in the levels of HSV- or CMV-specific IgG between HSK patients with and without CNV, and between those with different numbers of CNV quadrants or depths of corneal invasion ($p > 0.05$). We found HSV- and CMV-specific IgG to have no prognostic significance for the depth of corneal vascular invasion, the number of CNV quadrants or the presence of ulceration.

To summarize the above, D_{kl} score was used to assess the informativeness of the parameters. A small corneal curvature as assessed by keratometry was shown to be lowly informative ($D_{kl} = 0.11$ bit), whereas the presence of CNV and the depth of CNV, highly informative ($D_{kl} = 0.6$ bit and $D_{kl} = 0.9$ bit, respectively) for the diagnosis of corneal ulceration.

Discussion

HSK is a recurrent corneal immunoinflammatory disease that is characterized by chronic stromal inflammation and cell infiltration and neovascularization, with corneal ulceration being the most severe manifestation of the disease. Necrotizing stromal keratitis manifests as yellow-white infiltrations in the corneal stroma. Stromal CNV generally extends from the peripheral cornea to the central stroma. Chronic inflammation with stromal necrosis and ulcerative complications can result in corneal thinning, perforation and scarring, leading to visual loss [15]. Normally, the cornea is an avascular transparent ocular tissue with a regular optical surface. CNV impairs the optical properties of the cornea and leads to vision loss.

Table 4. Schirmer II and tear film break-up time (TBUT) test scores in HSK groups with no, one, two or three corneal quadrants affected by corneal neovascularization

Parameter	No CNV	One quadrant affected by CNV	Two quadrants affected by CNV	Three quadrants affected by CNV	P-value
	n = 12	n = 30	n = 14	n = 4	
	1	2	3	4	
Schirmer II test score (mm)	6.6 ± 1.7	6.8 ± 1.8	5.9 ± 1.5	6.2 ± 1.7	–
TBUT test score (s)	3.8 ± 1.1	2.9 ± 0.9	3.0 ± 1.0	2.0 ± 0.01	$p_{1–2} = 0.007$ $p_{1–3} = 0.04$

Note: CNV, corneal neovascularization; HSK, herpetic stromal keratitis; n, number of eyes; TBUT, tear film break-up time

The maintenance of corneal avascularity has been termed "angiogenic priviliage" [16].

CNV is a condition that can develop in response to inflammation, hypoxia, trauma or limbal stem cell deficiency. It is characterized by new blood vessels growing in from the corneal stroma and developing from pre-existing pericorneal vascular structures due to vascular endothelial cell proliferation and migration and into the corneal stroma. Histopathologically, new vessels were usually associated with corneal edema or inflammatory cells or both (76%) [17]. HSV infection shifts the balance of pro- and antiangiogenic factors toward pro-angiogenic status. Leakage of lipids into the stroma following herpetic CNV results in corneal opacification. Peripheral CNV associated with stromal scarring may, in rare circumstances, reduce vision by indirectly inducing astigmatism in the central cornea [16-18].

CNV reflects a multifactorial disease state sustained by chronic inflammation, extracellular matrix (ECM) remodeling, and dysregulated host-pathogen interactions [15]. CNV is tightly regulated by the balance between pro-angiogenic and anti-angiogenic factors. Key pro-angiogenic factors implicated in CNV include vascular endothelial growth factor A (VEGF-A), matrix metalloproteinases (MMP), angipoetins, platelet-derived growth factor (PDGF), hypoxia-inducible factors (HIF; tissue hypoxia can induce corneal angiogenesis [15]), inflammatory cytokines and chemokines, and ECM-associated regulators. Anti-angiogenic proteins include soluble VEGF receptor 1 (sVEGFR-1), pigment-epithelium-derived factor (PEDF), thrombospondins, yissue inhibitors of metalloproteinases (TIMPs), endostatin and anatomical and molecular barriers at the limbus that restrict vascular in growth. Shifting the balance toward pro-angiogenic signaling contributes to pathological vessel growth and progression of inflammation in the cornea [16, 19–21]. So far, immune-mediated tissue immune-mediated tissue damage has not fully explained the molecular mechanisms governing HSK progression toward CNV. Increasing evidence indicates that CNV results from complex interactions that extend beyond leukocyte-driven inflammation, as the host cell machinery, including key pathways and molecular markers, is hijacked by the invading virus to establish and perpetuate repetition and lifelong latency. These host–cell interactions regulate angiogenic imbalance, vascular privilege, and tissue remodeling, which collectively promote pathological vascular invasion [22].

Neovessels are characterized by altered vascular permeability and vessel maturation status, and contribute to inflammatory cell migration, edema and hypoxia. This creates a hostile microenvironment that induces the formation of new abnormal blood vessels [23]. In the current study, the PSV in the CRV was the lowest in the group without, and significantly increased by 7.2% in all groups with CNV. Hemodynamic forces are important regulators for maintenance, growth and regression of the vascular network. Under healthy conditions, endothelial cells are highly metabolically active, but mitotically quiescent. Increases in blood flow or the change fromlaminar to turbulent flow acts as an angioinductive signal onto the endothelium which is mediated by intra- and extracellular signaling cascades [24].

Therefore, increases in venous blood flow as a factor in CNV are likely to stimulate increases in the area of CNV. Pathological corneal angiogenesis in HSK contributes to the formation and chronicity of ulcerations because neovessel ingrowth increases inflammation, impairs epithelial regeneration and supports stromal destruction, and pathological angiogenic activity supports an inflammation cycle and may hamper effective healing. Additionally, ulcerative lesions induce the production of pro-angiogenic factors, forming the vicious cycle between tissue damage and pathological vessel ingrowth [23].

We found that the development of corneal ulcerations was accompanied by an increase in CNV area and the involvement of the deep corneal layers in CNV. The frequency of corneal ulceration increased with an increase in the number of CNV quadrants, showing a clear linear trend. This indicates that increased CNV area and the extension of CNV into the deep corneal layers resulted in an increased risk of epithelial and stromal damage, thus underlying the importance of early CNV progression control in HSK.

Findings of progressive HSK include hypoxia, increased hypoxia-induced expression of glycolytic genes, and increased lactate concentration in the cornea. In as study by Rao and Suvas [25], the magnitude of hypoxia correlated with the extent of neutrophils infiltrating the infected corneas. This is a local phenomenon which is difficult to identify individually under current clinical conditions. We also found that, in patients with HSK, systemic blood oxygenation was within the normal range and did not reflect the hypoxic processes in the cornea, which limited its informativeness.

Pachymetric and corneal epithelial thickness maps enable an objective evaluation of stromal inflammation and provide quantitative parameters for determining treatment strategy. Corneal thickness in HSK is characterized by phase changes that reflect inflammatory activity and tissue remodeling. In the acute phase of the disease, it increases to 560–650 μm and more locally due to stromal edema and infiltration. Corneal thickness in acute HSK rapidly decreases with treatment, with the decrease being correlated with the regression of inflammation. In chronic disease (especially necrotizing HSK), corneal thinning (to less than 450 μm) develops due to fibrous stromal remodeling and collagen loss. Corneal pachymetry (especially SD–OCT pachymetry) is a sensitive biomarker of disease activity and therapeutic response [26–28]. We found corneal thickness to range from 536 to 566 μm, which corresponded to the extent of corneal edema. This was in agreement with corneal densitometry measurements which indicated increased optical density of the cornea both in eyes with total corneal edema and those with focal

corneal edema, although the parameter was higher in the former eyes. No significant association was, however, found between these parameters and the presence of ulceration or CNV.

HSK is known to be accompanied by stromal remodeling (with stromal edema and/or scarring), especially when complicated by CNS, which results in changes in stromal curvature and formation of irregular astigmatism, as assessed by keratometry. No clear data on corneal curvature in HSK with both CNV and ulceration, however, has been reported until now. We found the corneal curvature in HSK with both CNV and ulceration to be significantly smaller than in HSK only. Additionally, the difference between maximum and minimum corneal epithelial thickness tended to increase in HSK patients with ulceration, with no significant difference between groups likely due to a large within-group variability caused by edema.

Corneal sensory neurons perceive mechanical, chemical and temperature stimuli and participate in tear and blink reflexes. Reduced corneal sensation (particularly, in herpetic keratitis) impairs reflex arcs, leading to tear secretion dysfunction and tear film instability. Because some patients with dry eye may have normal Schirmer test scores and low TBUT scores and others vice versa, the comprehensive evaluation of the results of both tests is critical for accurate diagnosis [29–31]. In a study on patients with unilateral quiescent HK [32], bilateral reduced tear secretion as assessed by Schirmer test and reduced TBUT scores were significantly correlated with corneal sensitivity in the eye with herpetic keratitis. Corneal sensitivity and TBUT were statistically lower in the affected eyes compared with the unaffected eyes in the HK group [33]. In the current study, Schirmer II test scores in patients with HSK were lower than the norm, with no significant difference between groups with various numbers of CNV quadrants, various depth of vascular invasion or the presence or absence of ulceration. Additionally, TBUT scores were lower than the norm, and were by 23.6% lower in HSK patients with one to three CNV quadrants than in those without CNV. To the best of our knowledge, no study has reported on the difference in Schirmer II values between HSK patients with various areas affected by CNV.

ELISA, polymerase chain reaction (PCR) and cell culture techniques are commonly used for assessing groups of HSK patients. Shoji and colleagues [34] performed measurement of HSV DNA by PCR, and of HSV-specific secretory IgA antibody, by ELISA in tears in patients with HSK. The overall sensitivity and specificity were 55.8 and 100 % for HSV DNA and 49.2 and 82.6 % for HSV-specific secretory IgA [34]. Serum HSV IgG/IgM antibody titers reflected the immune status and potential activity of HSV. They, however, showed no direct correlation with area or depth of corneal ulceration but rather reflected the possibility of the active process [35, 36]. In the current study, HSV IgG and CMV IgG showed no prognostic value for the depth of CNV, area of CNV or presence of ulceration (that is, the type of HSK).

Conclusion

In patients with HSK, the frequency of corneal ulceration increased with the area of CNV ($p = 0.0006$), and corneal ulceration risk was 4.3 times higher in HSK patients with mixed CNV than in those with superficial or deep CNV ($p < 0.001$). A small corneal curvature as assessed by keratometry was shown to be lowly informative ($D_{kl} = 0.11$ bit), whereas the presence of CNV and the depth of CNV, highly informative ($D_{kl} = 0.6$ bit and Dkl = 0.9 bit, respectively) for the diagnosis of corneal ulceration.

NVR was associated with changes in regional hemodynamics (particularly, with an increased CRV flow rate), and CRV flow rate was 7.2% higher in the presence of CNV ($p < 0.05$). A D_{kl} for the PSV in the CRV was 0.32 bit, indicating a moderate diagnostic value of the parameter and a relatively weak impact of the factor on hemodynamic parameters in the CRV.

The presence of CNV in HSK was associated with further worsening of Schirmer II and TBUT test scores, and TBUT test scores were by 23.6% lower in HSK patients with one to three CNV quadrants than in those without CNV ($p = 0.004$). CNS had a high diagnostic value ($D_{kl} = 0.77$ bit) for detecting abnormalities in tear secretion and tear film stability.

Author Contributions

MIR: Conceptualization, Methodology, Data Curation, Data Analysis, Writing – original draft preparation; KNI, Conceptualization, Writing – Review and Editing. All authors have read and approved the final version of the manuscript.

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Disclaimer

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Conflict of interest

The authors state that they have no conflict of interest that could influence their view on the subject matter or materials described and discussed in this manuscript.

Ethical statement

This study involved 60 patients (29 men and 31 women) with recurrent HSK, followed ethical standards as outlined in the Declaration of Helsinki of the World Medical Association and was approved by the Bioethics committee of SI “The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine” (Meeting Minutes No. 2 of April 18, 2025).

Informed consent

Informed consent was obtained from all subjects.

Data Availability Declaration

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

CMV, cytomegalovirus; CNV, corneal neovascularization; CRA, central retinal artery; CRV, central retinal vein; D_{kl}, Kullback–Leibler divergence; EDV, End-Diastolic Velocity; ELISA, enzyme-linked immunosorbent assay; HSV, herpes simplex virus; OA, ophthalmic artery; PSV, Peak Systolic Velocity; SpO_2, peripheral oxygen saturation by pulse oximetry; TBUT, tear film break-up time

References

1. Liesegang TJ, Melton LJ 3rd, Daly PJ, Ilstrup DM. Epidemiology of ocular herpes simplex. Incidence in Rochester, Minn, 1950 through 1982. Arch Ophthalmol. 1989 Aug;107(8):1155–9. doi: 10.1001/archopht.1989.01070020221029.
2. Park PJ, Chang M, Garg N, Zhu J, Chang JH, Shukla D. Corneal lymphangiogenesis in herpetic stromal keratitis. Surv Ophthalmol. 2015 Jan–Feb;60(1):60–71. doi: 10.1016/j.survophthal.2014.06.001. Epub 2014 Jun 10.
3. Chodosh J, Ung L. Adoption of Innovation in Herpes Simplex Virus Keratitis. Cornea. 2020 Nov;39 Suppl 1(1):S7–S18. doi: 10.1097/ICO.0000000000002425.
4. Farooq AV, Shukla D. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol. 2012 Sep;57(5):448–62. doi: 10.1016/j.survophthal.2012.01.005. Epub 2012 Apr 28.
5. McCormick I, James C, Welton NJ, Mayaud P, Turner KME, Gottlieb SL, et al. Incidence of herpes simplex virus keratitis and other ocular disease: global review and estimates. Ophthalmic Epidemiol. 2022 Aug;29(4):353–362. doi:10.1080/09286586.2021.1962919.
6. Young RC, Hodge DO, Liesegang TJ, Baratz KH. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976–2007: the effect of oral antiviral prophylaxis. Arch Ophthalmol. 2010 Sep;128(9):1178–83. doi: 10.1001/archophthalmol.2010.187.
7. Musa M, Enaholo E, Aluyi–Osa G, Atuanya GN, Spadea L, Salati C, Zeppieri M. Herpes simplex keratitis: A brief clinical overview. World J Virol. 2024 Mar 25;13(1):89934. doi: 10.5501/wjv.v13.i1.89934.
8. Kim GN, Yoo WS, Park MH, Chung JK, Han YS, Chung IY, et al. Clinical Features of Herpes Simplex Keratitis in a Korean Tertiary Referral Center: Efficacy of Oral Antiviral and Ascorbic Acid on Recurrence. Korean J Ophthalmol. 2018 Oct;32(5):353–360. doi: 10.3341/kjo.2017.0131.
9. Lobo AM, Agelidis AM, Shukla D. Pathogenesis of herpes simplex keratitis: The host cell response and ocular surface sequelae to infection and inflammation. Ocul Surf. 2019 Jan;17(1):40–49. doi: 10.1016/j.jtos.2018.10.002. Epub 2018 Oct 11.
10. Liesegang TJ. Classification of herpes simplex virus keratitis and anterior uveitis. Cornea. 1999 Mar;18(2):127–43. doi: 10.1097/00003226–199903000–00001.
11. Holland EJ, Schwartz GS. Classification of herpes simplex virus keratitis. Cornea. 1999 Mar;18(2):144–54. doi: 10.1097/00003226–199903000–00002.
12. Nanji A, Redd T, Chamberlain W, Schallhorn JM, Chen S, Ploner S, et al. Application of Corneal Optical Coherence Tomography Angiography for Assessment of Vessel Depth in Corneal Neovascularization. Cornea. 2020 May;39(5):598–604. doi: 10.1097/ICO.0000000000002232.
13. Zheng G, Gastwirth JL. On estimation of the variance in Cochran–Armitage trend tests for genetic association using case–control studies. Stat Med. 2006 Sep 30;25(18):3150–9. doi: 10.1002/sim.2250.
14. Spineli LM. Local inconsistency detection using the Kullback–Leibler divergence measure. Syst Rev. 2024 Oct 17;13(1):261. https://doi.org/10.1186/s13643–024–02680–4.
15. Wang L, Wang R, Xu C, Zhou H. Pathogenesis of Herpes Stromal Keratitis: Immune Inflammatory Response Mediated by Inflammatory Regulators. Front Immunol. 2020 May 13;11:766. doi: 10.3389/fimmu.2020.00766.
16. Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc. 2006;104:264–302.
17. Cursiefen C, Küchle M, Naumann GO. Angiogenesis in corneal diseases: histopathologic evaluation of 254 human corneal buttons with neovascularization. Cornea. 1998 Nov;17(6):611–3. doi: 10.1097/00003226–199811000–00008.
18. Abdelfattah NS, Amgad M, Zayed AA, Salem H, Elkhanany AE, Hussein H, Abd El–Baky N. Clinical correlates of common corneal neovascular diseases: a literature review. Int J Ophthalmol. 2015 Feb 18;8(1):182–93. doi: 10.3980/j.issn.2222–3959.2015.01.32.
19. Sharif Z, Sharif W. Corneal neovascularization: updates on pathophysiology, investigations & management. Rom J Ophthalmol. 2019 Jan–Mar;63(1):15–22.
20. Drzyzga Ł, Śpiewak D, Dorecka M, Wyględowska–Promieńska D. Available Therapeutic Options for Corneal Neovascularization: A Review. Int J Mol Sci. 2024 May 17;25(10):5479. doi: 10.3390/ijms25105479.
21. Ahmad A, Nawaz MI. Molecular mechanism of VEGF and its role in pathological angiogenesis. J Cell Biochem. 2022 Dec;123(12):1938–1965. doi: 10.1002/jcb.30344. Epub 2022 Oct 26. PMID: 36288574.
22. Akinsiku S, Shukla D. Molecular Pathways Driving Corneal Neovascularization in Herpes Simplex Keratitis. Pathogens. 2026 Feb 7;15(2):186. doi: 10.3390/pathogens15020186.
23. Giménez F, Suryawanshi A, Rouse BT. Pathogenesis of herpes stromal keratitis—a focus on corneal neovascularization. Prog Retin Eye Res. 2013 Mar;33:1–9. doi: 10.1016/j.preteyeres.2012.07.002. Epub 2012 Aug 7.
24. Schmidt VJ, Hilgert JG, Covi JM, Leibig N, Wietbrock JO, et al. Flow increase is decisive to initiate angiogenesis in veins exposed to altered hemodynamics. PLoS One. 2015;10(1):e0117407. doi:10.1371/journal.pone.0117407.
25. Rao P, Suvas S. Development of Inflammatory Hypoxia and Prevalence of Glycolytic Metabolism in Progressing Herpes Stromal Keratitis Lesions. J Immunol. 2019 Jan 15;202(2):514–526. doi: 10.4049/jimmunol.1800422. Epub 2018 Dec 7.
26. Wilhelmus KR, Sugar J, Hyndiuk RA, Stulting RD. Corneal thickness changes during herpes simplex virus disciform keratitis. Cornea. 2004 Mar;23(2):154–7. doi: 10.1097/00003226–200403000–00008.
27. Lu L, Palioura S. Management of Stromal Herpes Simplex Virus Keratitis With Epithelial Ulceration Using Opti-

cal Coherence Tomography–Generated Corneal Thickness Maps. Cornea. 2020 Dec;39(12):1566–1570. doi: 10.1097/ICO.0000000000002423.
28. Abtahi MA, Beheshtnejad AH, Latifi G, Akbari–Kamrani M, Ghafarian S, Masoomi A, et al. Corneal Epithelial Thickness Mapping: A Major Review. J Ophthalmol. 2024 Jan 2;2024:6674747. doi: 10.1155/2024/6674747.
29. Versura P, Giannaccare G, Pellegrini M, Sebastiani S, Campos EC. Neurotrophic keratitis: current challenges and future prospects. Eye Brain. 2018 Jun 28;10:37–45. doi: 10.2147/EB.S117261.
30. Messmer EM. The pathophysiology, diagnosis, and treatment of dry eye disease. Dtsch Arztebl Int. 2015 Jan 30;112(5):71–81; quiz 82. doi: 10.3238/arztebl.2015.0071.
31. Humayun S, Noor M, Shahid M, Naqvi SAH, Ishaq M, Humayun Q. Diagnosis of Dry Eye Syndrome Using Ocular Surface Disease Index, Tear Film Break–up Time, and Schirmer Test. J Coll Physicians Surg Pak. 2024 Mar;34(3):308–312. doi: 10.29271/jcpsp.2024.03.308.
32. Ma X, Lu Y. Bilateral tear film alterations in patients with unilateral quiescent herpes simplex keratitis. Acta Ophthalmol. 2017 Sep;95(6):629–633. doi: 10.1111/aos.13329. Epub 2016 Dec 9.
33. M'Garrech M, Rousseau A, Kaswin G, Sauer A, Barreau E, Bourcier T, Labetoulle M. Impairment of lacrimal secretion in the unaffected fellow eye of patients with recurrent unilateral herpetic keratitis. Ophthalmology. 2013 Oct;120(10):1959–67. doi: 10.1016/j.ophtha.2013.02.037. Epub 2013 May 9.
34. Shoji J, Sakimoto T, Inada N, Kamei Y, Matsubara M, Takamura E, Sawa M. A diagnostic method for herpes simplex keratitis by simultaneous measurement of viral DNA and virus–specific secretory IgA in tears: an evaluation. Jpn J Ophthalmol. 2016 Jul;60(4):294–301. doi: 10.1007/s10384–016–0448–y. Epub 2016 Apr 28.
35. Chen S, Peng Q, Wang H, Xie J, Cao H, Xu X. Seroprevalence and Clinical Insights of Ocular Herpesvirus Infections: A Cross–Sectional Study Evaluating ELISA as a Diagnostic Tool. Infect Drug Resist. 2025 Jun 19;18:3063–3070. doi: 10.2147/IDR.S527047.
36. Wang J, Cherfan DG, Goshe JM. Utility of HSV Serology for Chronic Corneal Pathology. Eye Contact Lens. 2020 May;46(3):190–193. doi: 10.1097/ICL.00.