Chapter

Recent Advances in the Diagnosis and Management of Herpetic Keratitis

Anna Nowińska

Abstract

The chapter is focused on one of the major cause of keratitis - Herpetic keratitis, its epidemiology, natural course, clinical forms, prognosis, diagnosis and treatment. The estimated global incidence of HSV keratitis is roughly 1,5 million, including 40,000 new cases of each year. Patients are usually affected in the early decades of live, therefore the disease has a severe impact on quality of life and quality of vision in young, productive adults. The author describes the detailed corneal characteristics, provides slit lamp photographs, optical coherence tomography scans and confocal microscopy results of different forms of the HSV keratitis: epithelial, stromal, necrotizing and endothelial. The chapter also discusses recent methods of diagnosis based on PCR testing as well as established and future methods of treatment based on the latest research results.

Keywords: HSV keratitis, Herpes simplex virus, confocal microscopy, optical coherence tomograpy

1. Introduction

Human herpesviruses, which include HSV-1 (Herpes simplex virus type-1), HSV-2 (Herpes simplex virus type-2), HZV (Herpes zoster virus), EBV (Epstein–Barr virus), CMV (Cytomegalovirus), HHV-6 (Human herpesvirus-6), HHV-7 (Human herpesvirus-7), HHV-8/KSHV (Human herpesvirus-8, Kaposi's sarcoma-associated herpesvirus) are the causative factor of various diseases, including mononucleosis, roseola, chickenpox and many forms of ocular involvement, such as conjunctivitis, blepharitis, keratitis, uveitis and retinitis. The common features of all human herpesviruses include a double-stranded DNA genome, a 20-faceted icosahedral capsid, a surrounding proteinaceous tegument, and an external glycoprotein-laden lipid envelope. All herpesviruses are able to achieve a state of the latency, where the virus remains inactive in cells and occasionally reactivates. Recurrence could be described as the most characteristic feature of corneal infections caused by HSV, subsequently leading to visual impairment and blindness. According to epidemiological data, HSV keratitis remains a leading infectious cause of blindness in the world. The estimated global incidence of HSV keratitis is roughly 1,5 million, including 40,000 new cases of each year. Additionally the recurrence rate is high. It was estimated as 9.6% at 1 year, 22.9% at 2 years, and 63.2% at 20 years after the first episode of documented HSV keratitis [1–4]. Also the worldwide seroprevalence rate is high and estimated above 50%, but recently it was reported declining in the United States [5].

In this chapter we will focus on Herpes simplex virus 1 keratitis - the detailed corneal characteristics based on slit-lamp examination, optical coherence tomography scans and confocal microscopy results. The chapter also discusses recent methods of diagnosis based on PCR testing as well as established and future methods of treatment based on the latest research results.

2. Pathogenesis of the HSV keratitis

General pathogenesis of herpesvirus infections include: active viral replication, state of latency and reactivation. Primary infection, usually in the childhood could be asymptomatic, oral, but also could affect upper respiratory track or ocular surface in the form of the conjunctivitis or blepharoconiunctivitis. After a primary infection, HSV-1 begins a life-long latency in the trigeminal ganglia, where abundant viral RNAs are constantly produced. In order to establish latency, HSV-1 has evolved several mechanism to evade the host immune response. The process is complex based on HSV-1 several viral proteins targeting multiple steps of the cellular DNA-sensor-mediated antiviral signal pathway of the host. Moreover, it is believed, that viral protein activation varies between immediate period after infection and the late phase of infection. Inhibition of the type I interferon (IFN-I) activity has been described as the main pathogenetic pathway of downregulating the host immune response. Numerous mechanism including: inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, modulating interferon regulatory factor 3 (IRF3), interferon regulatory factor 7 (IRF7) or stimulator of IFN genes (STING) function were identified. Recently a broad attention was brought to the HSV-1 immediate early (IE) protein infected-cell polypeptide 0 (ICP0), which is an E3 ubiquitin ligase, a nuclear phosphoprotein that was described to play an essential role in inhibition of IFN-I production through IRF7 protein expression reduction, thus promoting viral replication, latency, and reactivation. Certain triggering agents, physiological and environmental stress, including ultraviolet exposure, fever, injury, hormonal disruption or immunosuppression could cause viral reactivation in the tissues innervated by the trigeminal ganglion, causing different forms of the HSV keratitis: epithelial, stromal or endothelial. Epithelial keratitis is the most common form of HSV keratitis, but the recurrence infection may also affect other corneal layers. Recurrence varies in frequency between subjects and throughout the life and could cause irreversible corneal damage and decrease in visual acuity, ranging from superficial opacities to serious complications such as corneal perforation and endophthalmitis [6–10].

3. Diagnosis

The diagnosis of HSV keratitis is mainly based on the presence of typical unilateral corneal lesions on the slit lamp examination. However, the clinical diagnosis may be guided by modern imaging techniques, such as optical coherence tomography or confocal microscopy. Also, laboratory testing including polymerase chain reaction (PCR) and novel techniques based on multiplex dot hybridization (MDH) assay or immunochromatographic assay (ICGA) may serve as a potential guide in the diagnostic process.

3.1 Symptoms

Patients symptoms depend on the clinical form and stage of the disease. Primary infection may be asymptomatic. Recurrent infections symptoms include: foreign

body sensation, ocular or ocular adnexa pain, lacrimation, photophobia, decreased vision and conjunctival hyperemia. Symptoms are usually not specific. Although, patient with recurrent keratitis are aware of the symptoms of the recurrent keratitis, which allows for the rapid referral and treatment. Patients with neutrotrophic keratitis due to HSV keratitis may experience only mild symptoms despite the advanced corneal involvement.

3.2 Slit lamp examination/clinical forms

Herpetic keratitis is usually classified by anatomical localization in regards to affected corneal layers. Although the inflammation process may overlaps different layers. Also, recurrent keratitis is not only limited to one layer and can subsequently affect different corneal parts [11, 12].

3.2.1 Epithelial keratitis

Epithelial keratitis is the result of the active HSV replication in corneal, epithelial cells. The most characteristic form is the dendritic ulcer containing small branches with terminal bulbs. The borders of the branches are raised above the corneal surface. The ulcer may be single or multiple. Several dendritic ulcers may form a geographic ulcer, especially in patients with immune system deficiency, treated with topical steroids or in the long course of the disease. Other forms of epithelial involvement include punctate keratitis or epithelial vesicles. On the slit lamp examination, epithelial defects stain with fluorescein and become evident with the use of a blue filter (450 nm) with or without additional yellow barrier filter between 1 and 3 min after the dye instillation. Other symptoms in epithelial keratitis may include: bulbar conjunctival and limbal hyperaemia, subepithelial stromal edema at the ulcer site and subepithelial infiltration of inflammation cells. Epithelial keratitis in the form of the dendritic ulcer may also be present in the stromal recurrent keratitis. However, if multiple recurrence occur, the neurotrophic ulcer is definitely more probable clinical form compared to the dendritic ulcer. Characteristic features of the different forms of the epithelial keratitis are presented in **Figure 1**.

3.2.2 Stromal keratitis

Stromal involvement in case of herpetic keratitis develops on an immune related basis. Inflammatory response to the HSV is connected with the activation and infiltration of myeloid-derived cells, CD4+ T-cell and NK cells. Stromal inflammation may lead to the reduced corneal transparency, persistent scar formation, may also cause an irreversible tissue pathology including vascularization and stromal necrosis. The inflammation process is often accompanied by stromal localized or extensive corneal edema and a mild anterior chamber reaction. Several recurrences may lead to the lack of the corneal innervation. Moreover, the severity of disease may increase with each subsequent episode, as inflammatory reaction becomes stronger despite no detectable viral activity.

Throughout the years multiple clinical forms in terms of stromal keratitis were described, being the source of confusion in diagnostic terminology, including: immune stromal, interstitial, necrotizing, nonnecrotizing, disciform, focal, multifocal, diffuse. This could contributed to misdiagnosis, especially in early phases of the disease and misapplications of therapy in clinical practice. For example, in Japan, "disciform" keratitis is considered a type of stromal keratitis. "Immune stromal" term also is misleading, suggesting, that other forms of HSV stromal keratitis do not involve immune reaction. That is why a simplified classification of the stromal keratitis was

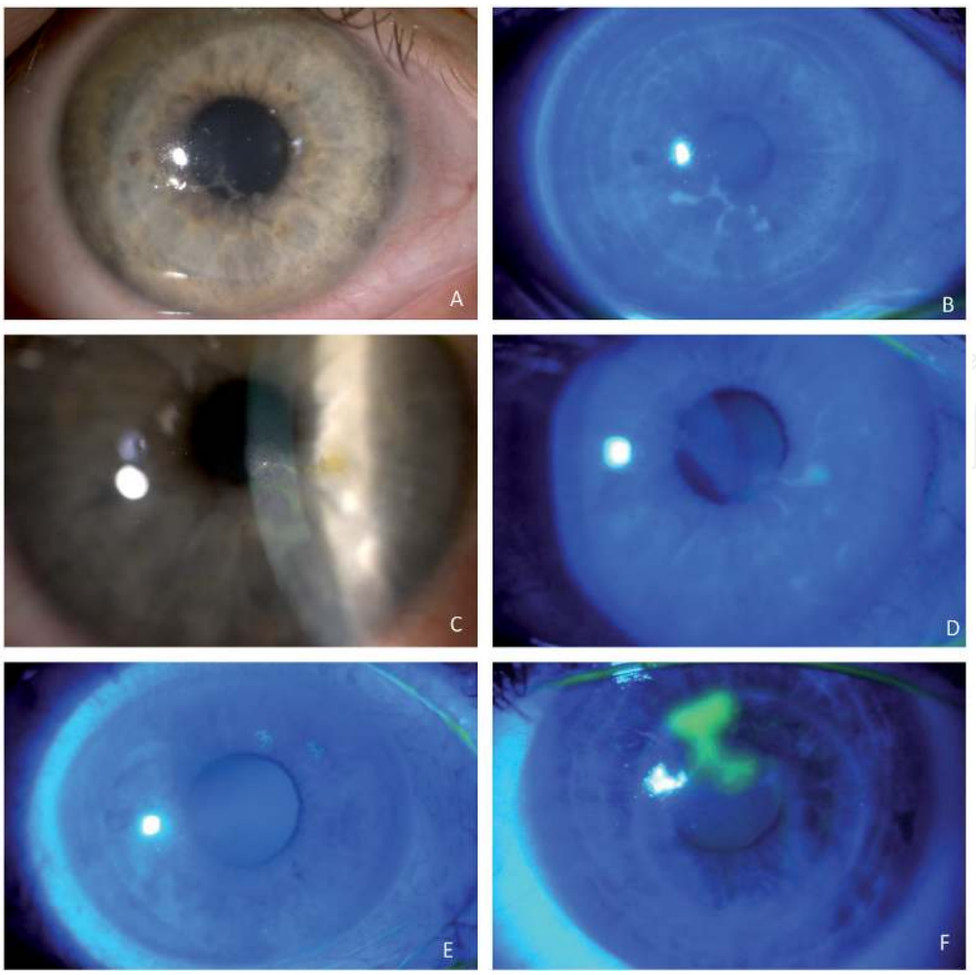

Figure 1.
Representative images of the slit-lamp photograph of the epithelial HSV keratitis. (A, B) Central, single dendritic ulcer before and after fluorescein installation. Branches with terminal bulbs visible. (C, D) Single dendritic ulcer with branches are raised above the corneal surface. Stromal haze accompanying the ulcer is noticeable. (E) Multiple, small dendritic ulcers visible under blue light after fluorescein installation. (F) Geographic, paracentral ulcer visible under blue light after fluorescein installation.

proposed, dividing the keratitis into two distinct forms: stromal with and without an overlying epithelial ulceration. Stromal keratitis without ulceration, is the more common form, historically described as "nonnecrotizing," "immune-stromal," and "interstitial." Stromal keratitis with ulceration is the effect of severe inflammation and relates to historical description of "necrotizing" keratitis. The form with the ulcer is more probably the result of stromal HSV reactivation, although the neurotrophic pathogenesis of the ulcer also cannot be ruled out. This terminology could be easily implemented in clinical practice and allows ophthalmologists to properly counsel patients regarding diagnosis, treatment and prognosis [12]. **Figure 2** contains clinical presentations of the range stromal keratitis. **Figure 3** present a clinical case of a patient diagnosed with stromal keratitis with ulceration throughout the treatment process.

Marginal keratitis is a special, rarely occurring form of stromal and epithelial keratitis. Clinically it is difficult to differentiate from other forms of marginal keratitis, thus laboratory testing may be helpful in establishing the final diagnosis. The lack of corneal sensitivity could also be used as a clinical clue in differential diagnosis.

3.2.3 Endothelitis

This form is believed to be a result of endothelial cells viral infection coexisting with immune reaction. Usually, the endothelitis is localized with a distinct area of

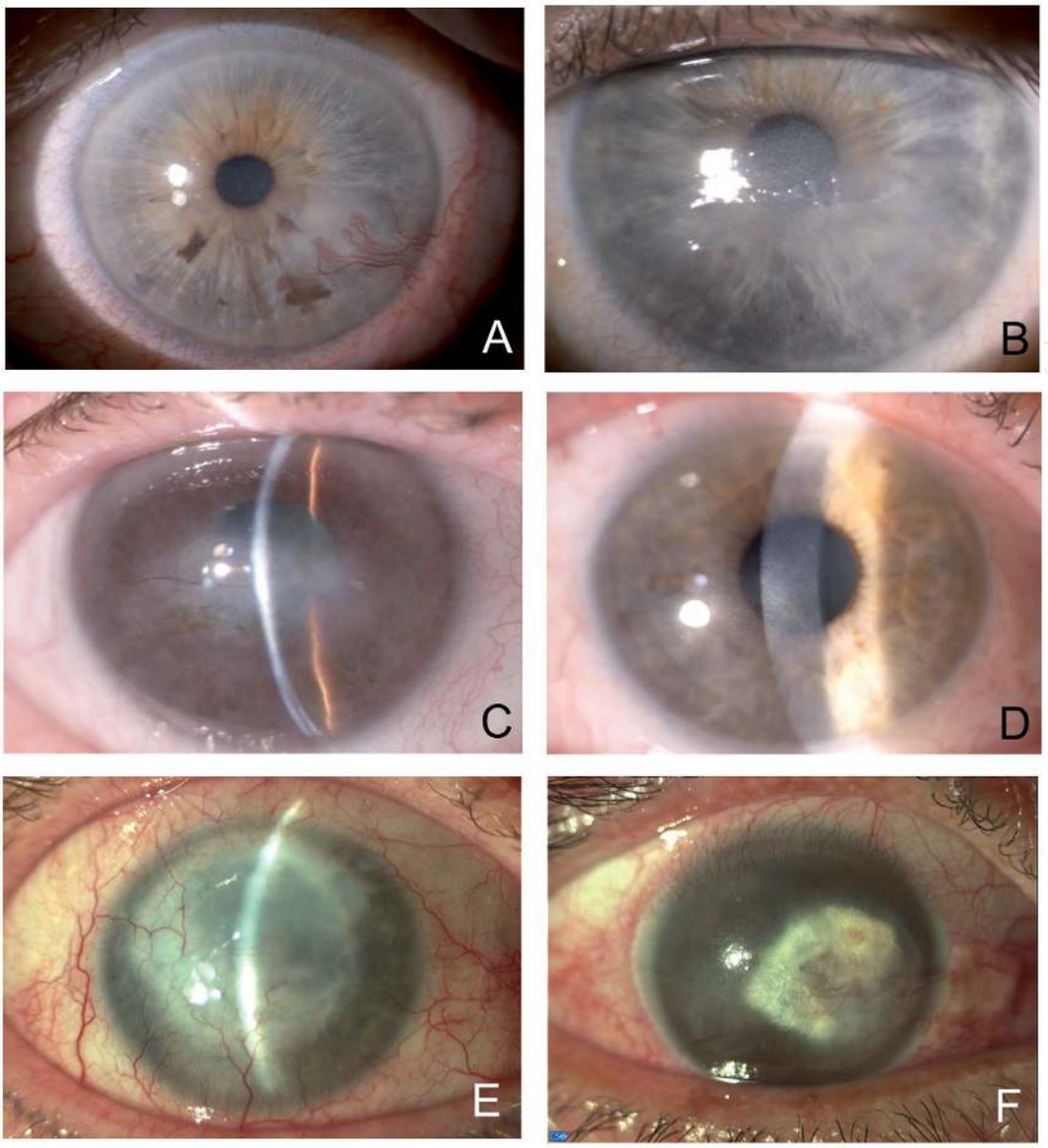

Figure 2.
Representative images of the slit-lamp photograph of eyes with the different involvement of the stromal keratitis or with corneal scars following HSV keratitis. (A) Paracentral stromal infiltration with profound, active limbal vascularization. (B) Epithelial, dendritic ulcer accompanied by active stromal keratitis with vascularization. (C) Central stromal scarring with deep, peripheral vascularization. (D) Stromal haze in the course of recurrent stromal HSV keratitis. (E) Excessive corneal scarring with significant, deep, peripheral vascularization. (F) Significant area of corneal scar accompanied by lipid keratopathy and deep vascularization.

the corneal edema. Therefore, it was historically described as disciform endothelial keratitis. Focal keratic precipitates, as well as Descemet membrane folds may be spotted in the affected area. Rarely, diffuse stromal edema, accompanied by trabeculitis with elevated intraocular pressure occurs. Various range of endothelitis is presented in **Figure 4**.

3.2.4 Neurotrophic ulcer/metaherpetic ulcer

This should be considered as a different entity, because there is no virus activation in case of neurotrophic ulcer. Also, the inflammation level compared to active HSV keratitis is lower. The most characteristic feature is the absence of corneal innervation and a non-healing corneal ulcer with smooth margins. As HSV keratitis alters the corneal nerves, the disease is one of the leading causes of neurotrophic keratopathy,

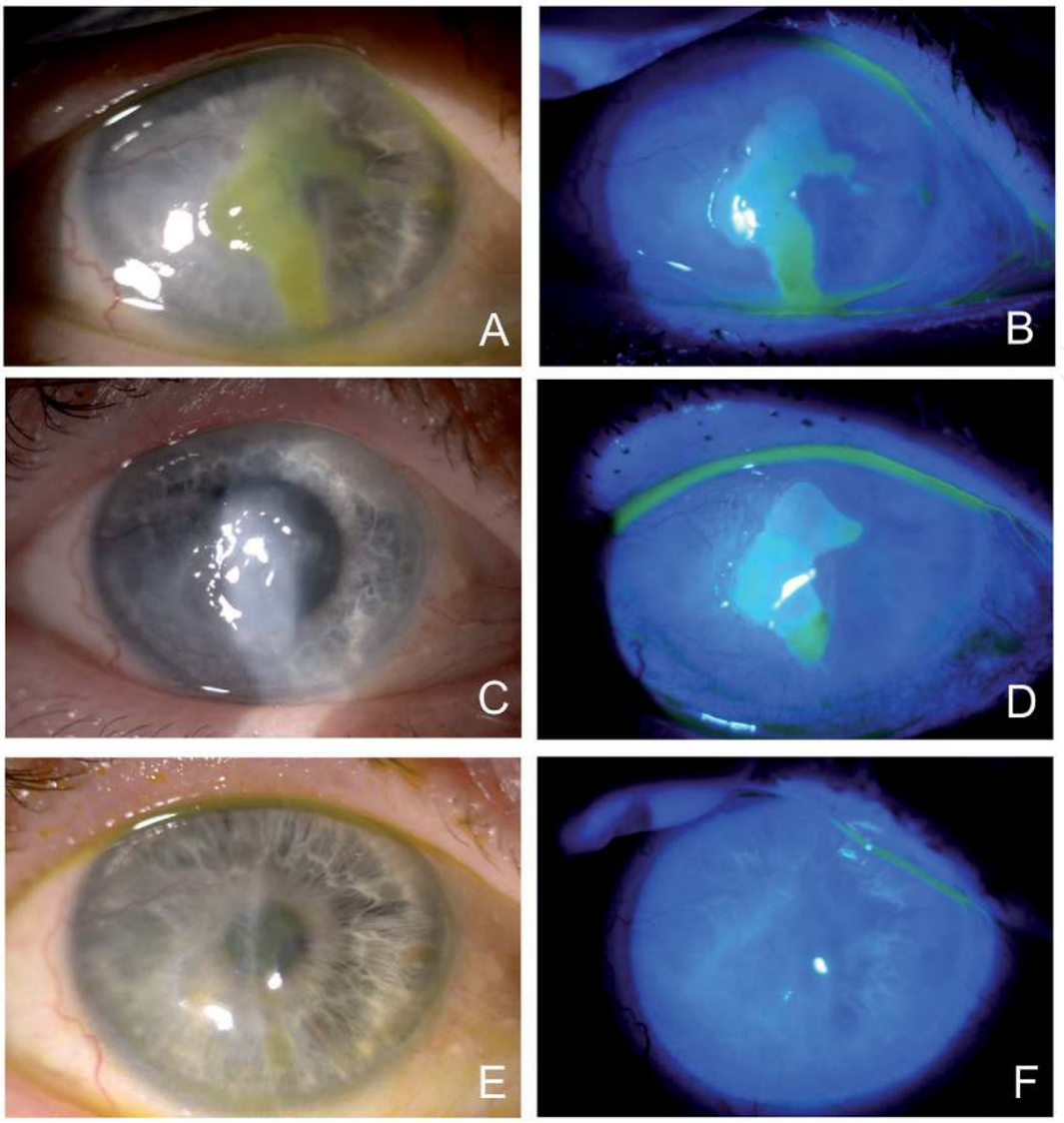

Figure 3.
Slit-lamp photographs presenting the follow up of a 65-year old patient with HSV stromal keratitis with ulcer. (A, B) Baseline, at diagnosis. Recurrent stromal keratitis with significant ulcer, stromal infiltration, vascularization and corneal thinning. Patient treated with the combination of antiviral medication (Oral acyclovir 800 mg, 5 times daily at baseline; topical 3% acyclovir ointment 4 times daily) combined with 0,1% dexamethasone (3 times daily) and preservative free lubricant eye drops (hourly). (C, D) At 1 month in the course of treatment. Significant decrease of the area of the ulcer. Remaining significant corneal infiltration with vascularization. Oral acyclovir dosage tapered gradually to 400 mg 4 times daily. Topical acyclovir discontinued. (E, F) At 3 months in the course of treatment. Ulcer healed completely. Punctate keratopathy visible under blue light. Decreased stromal infiltration, but stromal haze, thinning and vascularization visible. Oral acyclovir and 0,1% dexamethasone doses tapered very carefully within months to prevent active keratitis recurrence. Patient was recommended a frequent use of the preservative free eye lubricant drops.

among others, such severe dry eye disease, ocular burns or denervation post neurosurgical procedures. The pathogenesis is complex and include toxicity from antiviral medications, lack of nerve growth factors, the nerve damage as a result of recurrent keratitis. The neurotrophic keratitis is characterized by three stages of the severity: stage 1, punctate epithelial keratitis (PEK); stage 2, a nonhealing corneal persistent epithelial defect (PED); and stage 3 involving stromal involvement in the form of the neurotrophic ulceration. Possible accompanying signs are neovascularization, stromal haze and scarring. Consequently corneal poor ability to heal may result in corneal melting, prolonged ulceration, corneal perforation and endophthalmitis. A corneal sensitivity test is essential to confirm a diagnosis of neurotrophic keratitis. The test should be performed in regards to corneal location (central, peripheral), using a cotton-tipped swab or an esthesiometer. **Figure 5** presents forms of the neurotrophic keratitis.

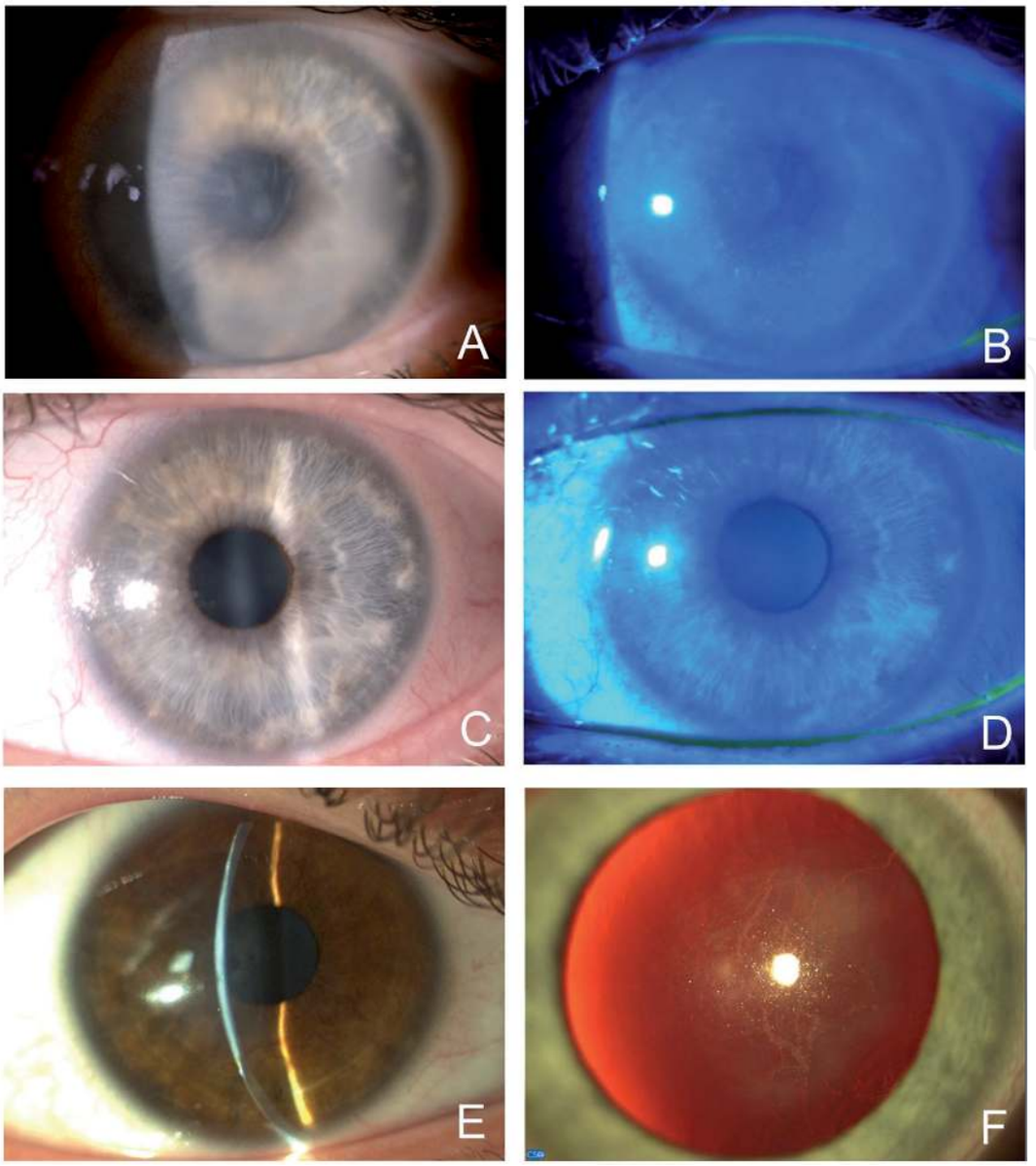

Figure 4.
Representative images of the slit-lamp photograph of the different forms of HSV endothelitis. (A, B, C, D) The slit lamp photographs of the eye of a 34-year old patient with recurrent, excessive endothelitis with significant corneal edema and Descemet folds. (A, B) At baseline. Diffuse corneal edema with Descemet folds and punctate keratopathy. Patient treated with the combination of antiviral medication (oral acyclovir 800 mg, 5 times daily at baseline combined with 0,1% dexamethasone (7 times daily) and preservative free lubricant eye drops (5 times daily). (C, D) At 2 months in the course of treatment. Significant decrease in stromal edema, with only subtle stromal haze. Improvement of the punctate epitheliopathy. (E) Distinct area of the corneal edema - disciform endothelial keratitis. (F) Distinct area of the corneal edema - disciform endothelial keratitis at retroillumination. Ghost, profound vessels visible.

3.3 Confocal microscopy

Confocal microscopy (IVCM - in vivo confocal microscopy) is the imaging technique developed to analyze corneal layers with the resolution of 1 μm. Imaging with confocal microscopy is used in clinical practice in differential diagnosis of microbial keratitis, corneal dystrophies and degenerations. The technique allows microscopic analysis of the cornea layer by layer and detailed assessment of keratocytes and inflammation cells. Features characteristic for HSV-1 keratitis depending on the stage and form include: microerosions, distortion of the superficial and basal epithelium, changes in superficial epithelial cell density, increase in epithelial cell size, squamous metaplasia, subepithelial infiltration of highly reflective dendritic

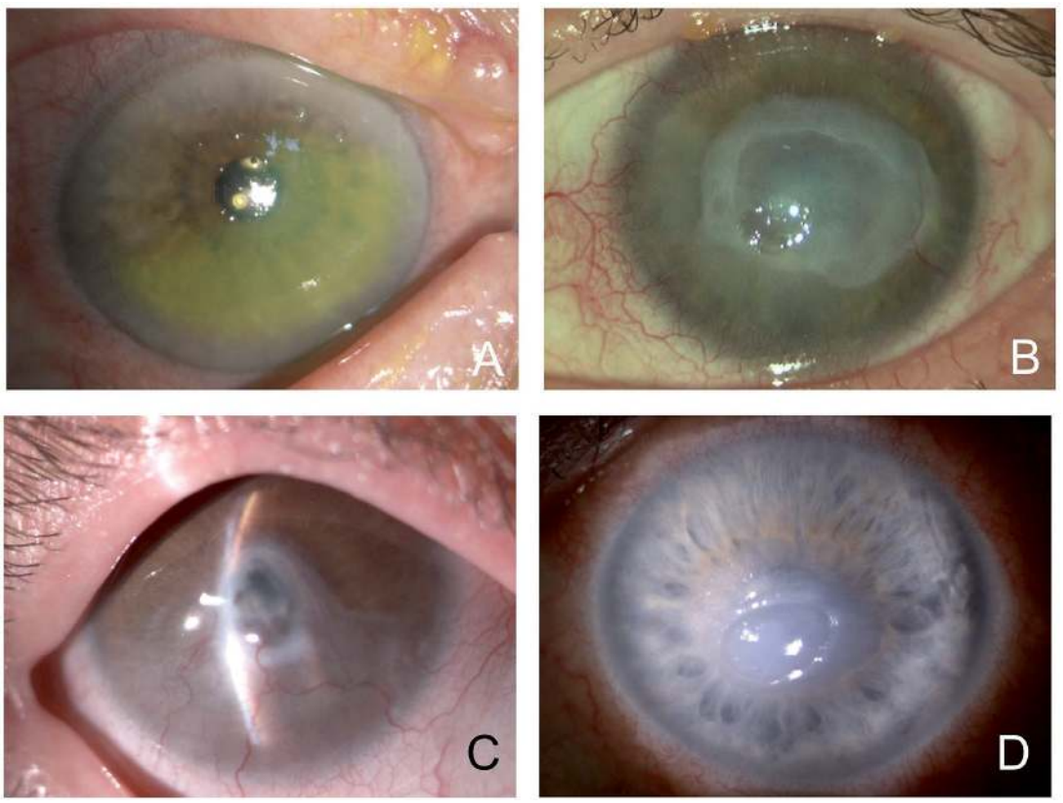

Figure 5.
Representative images of the slit-lamp photograph of the different forms of neurotrophic keratitis. (A) Neurotrophic keratitis stage 2. A nonhealing corneal persistent epithelial defect (PED) after fluorescein installation. (B) A single, central corneal ulcer with stromal infiltration and peripheral corneal vascularization. (C) Central corneal perforation in the course of the corneal thinning and scarring and vascularization. (D) Neurotrophic keratitis stage 3. Neurotrophic ulceration with elevated borders and significant stromal haze.

structures (correspondind to Langerhans cells), keratocytes activation, sub-basal nerve plexus alteration or absence, stromal fibrosis and endothelial precipitates. **Figure 6** presents the example of confocal microscopy results in case of patients with HSV keratitis. Confocal microscopy could guide in the disease diagnosis and monitoring the treatment results. In patients with stromal involvement the mean subbasal nerve density was proved to be significantly lower compared to healthy eyes. Also, in patients qualifying for surgical interventions, the technique has a potential role in assessing the sub-basal nerve plexus anatomy, helping the surgeons to procced with intervention decisions. The prognosis of patients with significantly altered corneal nerve plexus is poor after traditional transplant surgery [13–16].

3.4 Optical coherence tomography

Anterior eye segment imaging with 830 nm optical coherence tomography (AS OCT) was first demonstrated and published in 1994. Changing the light wavelength from 830 nm to 1310 nm allowed the direct transcleral anterior eye segment structures including trabecular-iris angle visualization in 2000. OCT provides in vivo anterior eye segment imaging with the axial resolution from 18 μm with time domain OCT (TD OCT) to 5 μm with spectral domain OCT (SD OCT) and to 5 μm with ultra high resolution spectral domain OCT. OCT is proven to provide reliable anterior eye segment morphology and morphometry results with high reproducibility and repeatability. Application of OCT in herpetic keratitis patients include: assisting in diagnosis of patients at active stage and assessing the scars in patients qualified for laser or surgical interventions. Active keratitis could be characterized

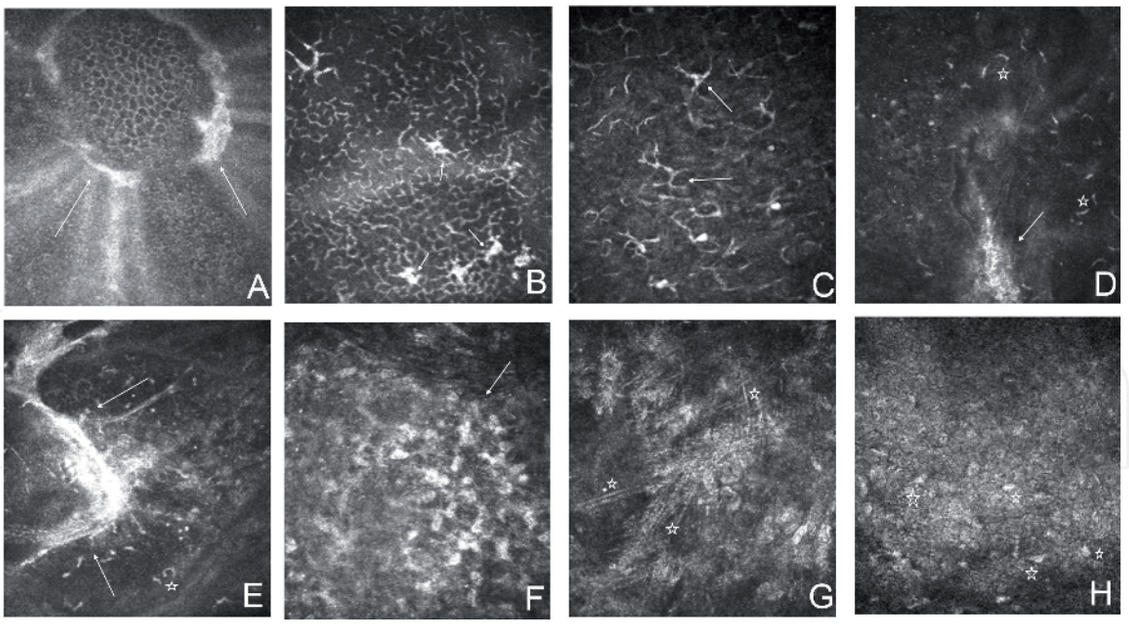

Figure 6.
Representative images of the confocal microscopy scans revealing significant features characteristic for HSV keratitis. (A) Epithelial, healed dendritic ulcer with noticeable fibrotic borders (arrows). (B) Multiple infiltration of small dendritic structures as the level of the epithelium. Clusters of inflammation cells (arrows). (C) Multiple infiltration of pronounced dendritic cells forming a lattice pattern (arrows) at the level of the basal epithelial cells. (D) Marked fibrosis at the level of the Bowman layer (arrow) with inflammation cells infiltration (stars). (E) Excessive fibrosis and inflammation cells infiltration forming clusters at the level of the Bowman layer (arrows). Dendritic structures visible (star). (F) Anterior stromal keratocytes activation with accompanying haze (arrow). (G) Stromal infiltration and haze accompanied by multiple crystalline structures due to the lipid degeneration (stars). (H) Multiple endothelial opacities. Examples marked with stars.

by the presence of the ulceration, stromal edema and inflammatory hyperreflective infiltrates. Corneas with inactive keratitis are characterized by stromal scarring and thinning, and epithelial remodeling [17–22]. Characteristic OCT features are presented in the **Figure** 7.

3.5 Laboratory testing

There are several laboratory techniques, which may help in the diagnostic process. Clinical samples for the analysis may be obtained through collection of tears, corneal epithelial cells, and conjunctival cells. Tear samples are usually obtained using Schirmer test. Epithelial or conjunctival cells may be collected through corneal scrapings, corneal impression membranes (CIM) or using conjunctival or corneal swab. The less invasive the technique the lesser probability of obtaining a clinically detectable material.

The isolation of the HSV from the cornea and performing a viral culture remains a conventional, gold standard technique, however the main disadvantages of this methods are low sensitivity and a time consuming process. Giemsa staining of the epithelial corneal cells may visualize multinucleated giant cells, resulting from coalescence of HSV infected epithelial cells and intranuclear HSV inclusions. Immunofluorescence assay (IFA) is one of the modern techniques developed to diagnose HSV keratitis. The principle of the method is to introduce antibodies, that bind to HSV antigens specifically to gain fluorescence based immunological detection of HSV-1 antigen through color visualization under microscopy. Disadvantages of the method include: required subjective interpretation by an experienced technician and the risk of obtaining false positives results due to cross-reactivity between other microorganisms.

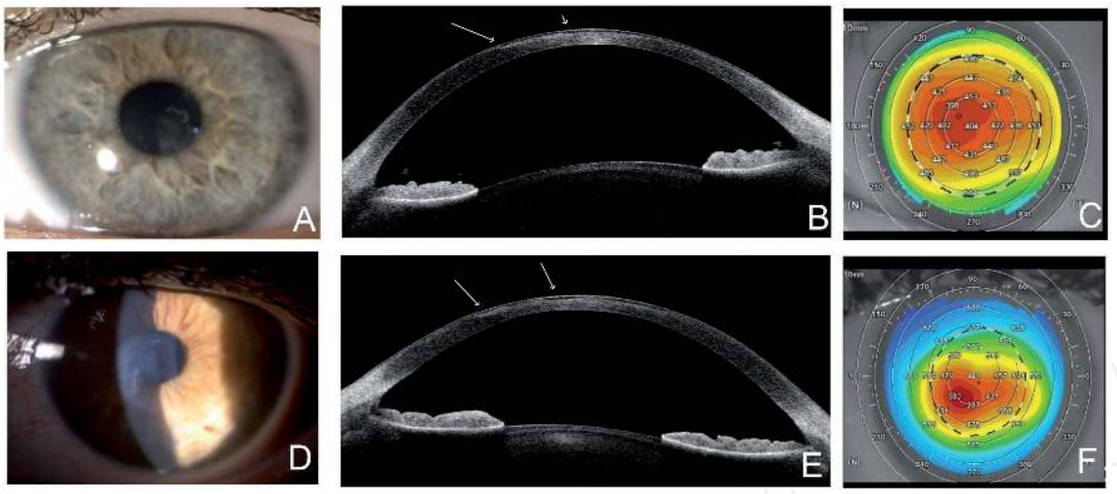

Figure 7.
Representative images of the anterior segment swept source optical coherence scans revealing significant features characteristic for HSV corneal scars. (A) Slit lamp photograph of the central post herpetic keratitis scar. (B) High resolution scan. Hyperreflective tissue within corneal stroma with irregular borders (arrows). (C) Pachymetry map. Marked paracentral corneal thinning to 398 μm. (D) Slit lamp photograph of the central post herpetic keratitis scar. (E) High resolution scan. Hyperreflective tissue within corneal stroma with irregular borders. Note the relatively smooth corneal surface and epithelial compensation over the irregular corneal stroma (arrows). (F) Pachymetry map. Marked irregular, paracentral corneal thinning to 382 μm.

Advanced diagnostic techniques include: Polymerase Chain Reaction (PCR) - conventional PCR, reverse transcriptase PCR (RT-PCR), real-time PCR (qPCR) and multiplex PCR. qPCR overcomes the disadvantages of conventional PCR by acquiring more rapid and sensitive results. Guda SJM. et al. assessed sensitivity and specificity of the conventional and real-time PCR compared to IFA performed on corneal scrapings. The sensitivity and specificity of conventional PCR was 100% and 76.9% and 100% and 28.2% of qPCR respectively. Satpathy et al. assessed and concluded, that specificity and positive predictive value (PPV) of PCR was higher in tear (90.6% and 37.5%). compared to cornea scrapings (71.3% and 30.3%). Moreover, Akbarian A. et al. reported, that conventional PCR with added internal amplification control (IAC) had higher sensitivity (100%) vs. culture method (66.66%), while the specificity was 100% for both diagnostic methods.

Also novel methods, such as multiplex dot hybridization (MDH) assay, immunochromatographic assay (ICGA, AmpliVue) or Infected cell protein 0 (ICP0) detection in tears are either tested or incorporated into a clinical practice. AmpliVue is a commercially available immunochromatographic assay, office-based diagnostic test characterized by a 64.7% positive detection rate. Sensitivity and specificity of AmpliVue was assessed as 84% and 100% respectively, based on true positives from culture and PCR combined. The MDH assay is a rapid technique, that involves a series of oligonucleotide probes specific for HSV genes. Compared to the real-time PCR, the MDH assay is characterized by very high values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 93.3%, 100%, 100% and 98.4%, respectively. The infected cell protein 0 (ICP0) is an acute phase protein during HSV infection and plays a significant role in the virus gene expression activation. ICP0 could be potentially defected in tears of affected subjects [23–28].

4. Differential diagnosis

Differential diagnosis is dependent on the corneal layer affected by the keratitis. Epithelial keratitis should be differentiated with epithelial regeneration line

after traumatic epithelial defect, epithelial corneal dystrophies, such as epithelial basement membrane corneal dystrophy (EBMCD; map-finger-dot dystrophy, Cogan microcystic dystrophy), epitheliopathy associated with excessive contact lens wear or iatrogenic epitheliopathy after topical drops containing preservatives. Stromal involvement requires differentiation with other microbial keratitis (bacterial, fungal or amoebic), vaccinia virus keratitis (VACVK), Varicella Zoster virus keratitis, Thygeson superficial punctate keratopathy, stromal or Bowman layer corneal dystrophies, such as TGFBI corneal dystrophies. Marginal keratitis should be differentiated with other forms of marginal ulcers, such as staphylococcal marginal keratitis or related to atopic or autoimmune diseases, such as rheumatoid arthritis, systemic lupus or granulomatosis with polyangiitis (GPA). Also, neurotrophic keratitis may be initiated by multiple other causes, such as surgical and laser procedures, chemical burns, excessive contact lens wear and preservative-containing topical medicines, diabetes mellitus, multiple sclerosis and congenital or acquired abnormalities of the trigeminal nerve. Examples of the diseases, which require differential diagnosis with HSV keratitis are presented in **Figure 8**.

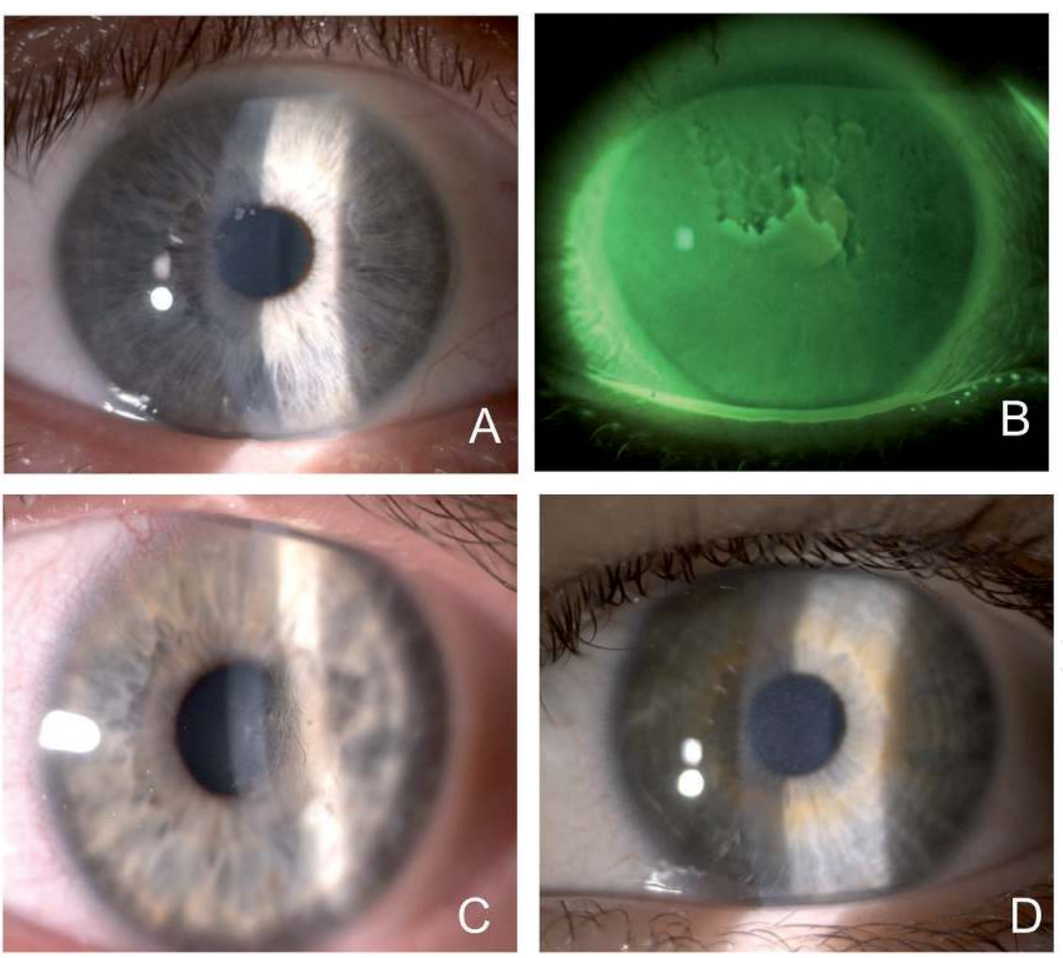

Figure 8.
Representative images of the slit-lamp photograph of the different forms of corneal diseases, which should be differentiated with HSV-keratitis. (A) Slit-lamp photograph of the epithelial basement membrane corneal dystrophy (EBMCD; map-finger-dot dystrophy, Cogan microcystic dystrophy). Superficial white dots visible. (B) The slit lamp photograph after fluorescein installation under blue light with additional yellow barrier filter of the patient 9A. An irregular area of the disrupted epithelium visible. (C) Slit-lamp photograph of the lattice corneal dystrophy (LCD). A dystrophy was confirmed by the TGFBI gene testing, which revealed a H626R mutation. (D) Slit-lamp photograph of the pediatric form of the lattice corneal dystrophy (LCD). Epithelial haze with multiple small, gray dots is visible. A dystrophy was confirmed by the TGFBI gene testing, which revealed a R124C mutation.

5. Management

Major advances in the treatment of HSV keratitis have been provided by the evidence-based results and conclusions of the Herpetic Eye Disease Study (HEDS) randomized clinical trials, which were multicenter, characterized by double-masking with placebo controls studies. Based on this knowledge, further treatment guidelines were proposed and published [12, 29–37]. Although the HEDS clinical trials directly addresses multiple clinical concerns, the studies have also several limitations. These include: inadequate sample size in case of HSV stromal keratitis with epithelial ulceration to determine the optimal course of therapy, relatively high rate of follow up failure within the study group. Also, the corticosteroid regimen was standardized and fixed in the study group, thus lacking the evidence of benefit of delivery of personalized care. Finally, the concerns regarding the dose and the optimal period of antiviral prophylaxis have not been resolved.

5.1 Active keratitis

Nowadays, the main treatment line of the active keratitis is a combination of the antiviral and corticosteroids drugs, depending on the epithelial and stromal involvement. The general rule to follow is to avoid corticosteroids in epithelial keratitis, because the entity of this form is virus activation and to treat with corticosteroids in stromal and endothelial keratitis without epithelial involvement, because those forms are strongly connected with the significant reaction of the immune system.

Antiviral drugs are used in two main forms: topical and oral. Topical anti HSV-1 drugs include: trifluridine solution (1%), ganciclovir gel (0.15%), and acyclovir ointment (3%). Oral anti HSV-1 drugs include: acyclovir, valacyclovir, and famciclovir. Historically, other systemic drugs were also used, such as idoxuridine, vidarabine, valganciclovir, foscarnet, and cidofovir, but they were withdrawn from the market or are relatively too toxic in combination with the achieved therapeutic effect.

Most common antiviral drug worldwide is acyclovir used either orally or topically or in combination. Common side effects of the prolonged oral acyclovir include nausea, vomiting, diarrhea, headache and weakness. Potentially serious, but very rare side effects include renal failure and hematology complications, such as: thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS).

In recent years, there have been an increasing interest in valacyclovir, due to its proven improved bioavailability and steadier plasma concentration compared to acyclovir. Valacyclovir is considered a prodrug of acyclovir. The lower frequency of dosing (2 times daily versus 5 times daily) may be a strong benefit for some patients. However, there is a lack of strong evidence, that treatment with valaciclovir provides leads to better results and less ocular and systemic complications. Comparing to herpes zoster ophthalmicus, the authors of the systematic Cochrane report indicated uncertainty of the relative benefits and harms of valacyclovir over acyclovir [38]. All topical antiviral drugs are characterized by ocular surface toxicity, could cause allergic reactions, and punctal and nasolacrimal duct stenosis, therefore the prolonged usage of those formulas is not advised. Authors of the Cochrane systematic review on HSV keratitis treatment, published in 2015 also assessed other methods of HSV keratitis treatment, such as manual debridement of the corneal epithelium or experimental biologic agents. Manual debridement alone has been proved to be not effective. Also, topical treatment with interferon has only a modest benefit over placebo [39]. In case of epithelial keratitis, the mainstay of treatment is antiviral agents. Corticosteroids excessive usage may lead to geographical ulcers

and delay healing of the epithelium. When stromal involvement is present, the mainstay of treatment is the use of corticosteroids with the combination of antiviral agents. The HEDS clinical trials have brought solid rationale for the treatment of the stromal keratitis with corticosteroids. Nowadays, there are several available topical corticosteroids formulas with different anti-inflammatory potency and different potential for adverse reactions: Dexamethasone 0,1%, Betamethasone at concentrations ranging from 0.01% to 0.1%, Prednisolone 1%, Loteprednol Etabonate 0.5%, Rimexolone 1%, Dexamethasone 0.1%, Hydrocortisone 0.335%, Fluorometholone 0,1%. The strongest anti-inflammatory effect is demonstrated by dexamethasone, the weakest by hydrocortisone. This should be taken into consideration, when choosing the medication depending on the level of the corneal inflammation. Moreover, steroid medication must be withdrawn gradually, tapering the doses generally over few weeks' time. During the drug withdrawal, instead of sudden discontinuation of the stronger corticosteroid, one may consider replacing it with a relatively weaker one to avoid a rebound increase in inflammation and a disease recurrence immediately after drug cessation. The recommended treatment for HSV stromal keratitis without ulceration should include a topical corticosteroid for at least a period exceeding ten weeks in conjunction with a prophylactic oral antiviral. A treatment period greater than ten weeks has been recommended, because of the high treatment failure rates six weeks after a ten-week prednisolone taper in the HEDS clinical trial. The most concerning side effects of topical steroids include: increase of the intraocular pressure, cataract and secondary infections (including bacterial, fungal, and also viral infections). Therefore, patients must be monitored carefully when treating with topical steroids.

5.2 Recurrence prevention

The HEDS study on recurrence rate clearly demonstrated that short-course oral during an active HSV epithelial keratitis does not prevent later stromal keratitis or iritis. On the other hand, a 12-month course of prophylactic oral acyclovir (400 mg) twice daily significantly decreased a recurrence rate of the stromal involvement. Although the HEDS study authors did not recommend a prolonged, beyond 12 months acyclovir prophylaxis, clinical practice recommendations and observations seem to postulate a positive role of a long-term prophylaxis, especially in patients with a high recurrence rate, significant corneal thinning at risk of corneal perforation, with comorbidities, such as atopy, autoimmune diseases or in immunocompromised patients. Also patients with history of HSV keratitis undergoing surgical procedures, such as corneal transplant, photorefractive procedures or cataract surgery may benefit from acyclovir prophylaxis, until the level of inflammation associate with the procedure and the risk of recurrence is decreased [12, 29–37].

One of the future treatment strategies is to enhance patient's immune system resistance to the infection through a vaccine against HSV-1. Nowadays there are no approved vaccine available, but there are ongoing studies regarding this subject. In the recently published study in 2020, the authors identified 15 viral-encoded proteins, which could serve as candidates for further testing for the HSV-1 vaccine [40].

5.3 Neurotrophic keratitis

There are several methods of treatment depending on the severity level of keratitis. First line therapy includes discontinuing potentially toxic topical medications, tear replacement products and oral supplementation with omega-3 fatty acids. The next step of treatment is immunomodulatory therapy including:

lifitegrast, cyclosporine and steroids at different frequency and concentrations, and also autologous serum eye drops at concentrations from 20–100%. Autologous serum eye drops are characterized by multiple benefits: biochemical characteristics, including pH, nutrient content, vitamins, fibronectin, growth factors such as epithelial growth factor (EGF) or nerve growth factor (NGF), are similar to that of human tears, the serum eye drops also inhibit the release of inflammatory cytokines and increase the number of goblet cells and mucin expression in the conjunctiva. Prolonged use of serum eye drops is proved to restore homeostasis of the ocular surface.

In the last few years, there have been an increasing interest in the implementation of the Nerve Growth Factor (NGF) in the sub-basal nerve plexus regeneration, leading to the complete healing of the neurotrophic ulcers. NGF is an endogenous protein involved in the differentiation and maintenance of all systemic neurons, while in corneal tissue it is established to play a role in corneal innervation, tear secretion mechanism, and corneal epithelial cell growth and stability. Cenegermin is a recombinant human Nerve Growth Factor (rhNGF) that is structurally identical to the human NGF protein made in ocular tissues, it was introduced in the ophthalmic solution at concentration of 0.002% (20 mcg/mL). Two controlled clinical trails in Europe (REPARO) and USA (NGF0214) provided strong evidence on its effectiveness. 72% and 65% of patients with neurotrophic keratitis receiving cenegermin were completely healed in Europe and USA trails respectively [41–44]. Matrix regenerating agent (ReGenerating Agent; RGTA), mimicking natural heparan sulfate within the corneal tissue, is also a recent topical agent showing promising results in the treatment. RGTA eye drops (Cacicol; Thea) are preservative-free, well-tolerated, proved to promote regeneration of damaged tissues and to enhance corneal tissue healing [45, 46].

Novel emerging treatment approaches also include thymosine β4, CODA001, topical insulin, Substance P and insulin-like growth factor 1 (IGF-1). Thymosine β4 and CODA001 are in the most advanced evaluation undergoing clinical trials. Thymosin beta 4 is a 43-amino acid peptide, a major constituent protein of macrophages, and platelets. Currently, third-phase, multi-center, randomized, double masked, placebo controlled clinical study is ongoing regarding its role in ocular surface healing. Insulin at 3 different concentrations. CODA001 is an antisense oligonucleotide (antisense deoxynucleotide oligomer) that modulates and down-regulates the expression of the gap junction protein Cx43 (Connexin-43), which is increased in persistent epithelial defects [47].

Other procedures implemented at different severity levels of neurotrophic keratitis include: therapeutic contact lenses, lacrimal punctual occlusion, amniotic membrane contact lens or transplantation, partial or complete tarsorraphy, corneal transplant, conjunctival flap transplant or direct neurotization.

Amniotic membrane transplantation (AMT) is proved to provide many benefits in the treatment of neurotrophic keratitis. AMT inhibits the activity of inflammatory cells, extends the life of corneal epithelial stem cells and maintains their ability to regenerate epithelial cells, promotes healing of the corneal wounds, blocks the TGF-ß cytokine system activation and the transformation of fibroblasts into myofibroblasts, also creates a protective membrane covering the affected ocular surface tissues. In dry eye disease, it is used in case of serious complications, such as corneal ulcer or microperforation. An interesting solution to consider is a sutureless, adhesiveless amniotic membrane transplant (AMT; ProKera; Bio-Tissue, Inc.) implantation. It is a corneal–epithelial device that consists of a polycarbonate ring conformer containing cryopreserved amniotic membrane. Advantages of this design include: shorter surgical time and prevention of suture-related complications [48].

To summarize neurotrophic keratitis treatment: a stepwise approach should be implemented with careful exclusion of the active infection. Topical treatments should be the first line therapy over the surgical interventions.

5.4 Surgical interventions

Surgical interventions in active HSV keratitis are limited to the severe stromal involvement with the increased risk of corneal perforation. Those may include: application of cyanoacrylate glue, amniotic membrane transplantation or therapeutic keratoplasty.

Other indications for surgical procedures include inactive corneal scarring after keratitis or cataract formation mainly due to prolonged treatment with topical steroids. Superficial opacifications could be considered as an indication for phototherapeutic keratectomy (PTK), although the corneal thinning is usual after HSV keratitis and therefore it limits the use of this method. The PTK ablation should always be limited to anterior one-third of stromal layers and leave a minimum residual stromal bed thickness (RSBT) of 250 μm to avoid further corneal ectasia. Also, spontaneous reactivation of HSV keratitis is well known after PTK, because laser ablation stimulates viral shedding in tears and reactivates the virus [49, 50].

When an extensive scar with corneal thinning is present a deep anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK) should be considered. DALK eliminates the risk of endothelial immunologic rejection, but due to advanced corneal scarring and thinning may be difficult to perform. An obligatory preoperative assessment before keratoplasty procedures include the corneal sensitivity analysis and the exclusion of the active viral infection with neovascularization. It is well established, that the presence of deep stromal vascularization exceeding 2 or more quadrants, creates a significant risk for a graft immunologic rejection and graft failure. Another factor, strongly connected to the increased risk of the graft failure is a herpetic infection recurrence. To address those issues, the combination of the antiviral prophylaxis with the prophylaxis of a immunologic rejection should be implemented. Antiviral prophylaxis includes the use of high-dose oral acyclovir as recommended by American Academy of Ophthalmology (AAO guidelines recommended 800 mg 3 times daily for at least 1 year) [37]. The prophylaxis of a immunologic rejection includes usually systemic steroids combined with topical therapy. Despite the prophylaxis, there is a relatively high rate of graft failure performed in eyes after herpetic keratitis reported in the literature: 26% at 3 years, 15% at 5 years and 53.7% at 8 years [51–53]. In the last years, there have been an increasing interest in keratorosthesis surgery, as a viable option allowing a long term restoration of vision in patients with high risk for corneal transplantation. Boston type I keratoprosthesis (BKPro) is the most commonly implanted keratoprosthesis worldwide. BKPro was first used in 1965 by Professor Claes H. Dohlman [54, 55]. The BKPro surgery is usually complex with the high incidence of intraocular complications. Also the rate of postoperative complications is high and includes: glaucoma, retroprosthetic membrane formation, keratolysis, endophthalmitis, vitreoretinal complications, such as retinal detachment, cystoid macular edema, uveitis and hypotony/phthisis. In the latest study of the long term BKPro outcomes published in 2020, the probability of maintaining or improving vision was 75,0% at 5 years and 66,7% at 10 years [56].

In summary, surgical intervention in HSV keratits is challenging and high-risk procedure, therefore a special attention should be brought when referring such patients.

6. Conclusions

HSV keratitis due to its multiform occurrence remains a challenging diagnostic in clinical practice. Modern imaging technique, such as optical coherence tomography or confocal microscopy as well as modern laboratory testing including multiplex dot hybridization (MDH) assay, immunochromatographic assay (ICGA, AmpliVue) are useful in guiding the diagnostic process.

Ocular surface homeostasis should be always considered when treating HSV keratitis, especially in the neurotrophic keratitis at different severity grades.

Conflict of interest

The author has no conflict of interest.

Author details

Anna Nowińska[1,2]

1 Chair and Clinical Department of Ophthalmology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland

2 Ophthalmology Department, Railway Hospital in Katowice, Poland

*Address all correspondence to: anna.nowinska@sum.edu.pl

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