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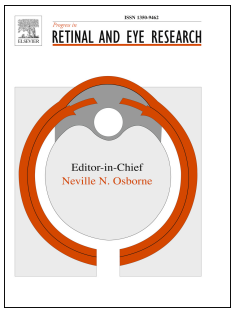

Neuropathic keratopathy

Harminder S. Dua, Dalia G. Said, Elisabeth M. Messmer, Maurizio Rolando, Jose M. Benitez-del-Castillo, Parwez N. Hossain, Alex J. Shortt, Gerd Gerling, Mario Nubile, Francisco C. Figueiredo, Saaeha Rauz, Leonardo Mastropasqua, Paolo Rama, Christophe Baudouin

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Declaration of Interests

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Title: Neuropathic Keratopathy

Contents:

Abbreviations

AMT	Amniotic membrane transplant
ASOCT	Anterior Segment Optical Coherence Tomography
BAK	Benzalkonium chloride
BNGA	Belmonte non-contact gas aesthesiometer
CGRP	Calcitonin gene-related peptide
IVCM	In Vivo Confocal Microscopy
LC	Langerhans cells
MMP	Matrix metalloproteases
NGF	Nerve Growth Factor
NK	Neurotrophic Keratopathy
PED	Persistent epithelial defect
SCG	Superior Cervical Ganglion
SPK	Superficial punctate keratitis
TRP	Transient Receptor Potential
VEGF	Vascular Endothelial Growth Factor

Abstract

Neurotrophic Keratopathy (NK) refers to a condition where corneal epitheliopathy leading to frank epithelial defect with or without stromal ulceration (melting) is associated with reduced or absent corneal sensations. Sensory nerves serve nociceptor and trophic functions, which can be affected independently or simultaneously. Loss of trophic function and consequent epithelial breakdown exposes the stroma making it susceptible to enzymatic degradation. Nerve pathology can range from attrition to aberrant re-generation with corresponding symptoms from anaesthesia to hyperaesthesia/allodynia. Many systemic and ocular conditions, including surgery and preserved medications can lead to NK. NK can be mild (epithelium and tear film changes), moderate (non-healing epithelial defect) or severe (stromal melting and perforation). Moderate and severe NK can profoundly affect vision and adversely impact on the quality of life. Medical management with lubricating agents from artificial tears to serum/plasma drops, anti-inflammatory agents, antibiotics and anti-proteases all provide non-specific relief, which may be temporary. Contact lenses, punctal plugs, lid closure with botulinum toxin and surgical interventions like tarsorrhaphy, conjunctival flaps and amniotic membrane provide greater success but often at the cost of obscuring sight. Corneal surgery in a dry ocular surface with reduced sensation is at high risk of failure. The recent advent of biologicals such as biopolymers mimicking heparan sulfate; coenzyme Q10 and antisense oligonucleotide that suppress connexin 43 expression, all offer promise. Recombinant nerve growth factor (cenegermin), recently approved for human use targets the nerve pathology and has the potential of addressing the underlying deficit and becoming a specific therapy for NK.

Key Words:

1. Keratitis
2. Neurotrophic Keratopathy
3. Trigeminal Neve diseases
4. Matrix Regenerating agents
5. Nerve growth factor

1. Introduction

The ocular surface is a continuous epithelium and underlying stroma extending from the muco-cutaneous junction at the eye lid margin to the corneal surface. It is a specialised system that closely interacts with associated adnexal structures, lacrimal glands and eyelids (known as the lacrimal functional unit), and via cross-talk with the neural, endocrine, vascular, and immune regulatory systems (Gipson, 2007). Aragona and Rolando described the ocular surface unit as a dynamic complex that includes the eye lids, tear film, conjunctiva and cornea functioning as a single unit, where one reflects and influences the others (Aragona and Rolando, 2013). It represents the interface between the external environment and the eye. Its key function is to guarantee a clear optical surface to direct light to the retina, a neural tissue inside the eye that signals to the brain for visual recognition. It also provides protection for the inner structures of the eye. To do this it must be able to quickly adapt to the changing conditions of the environment or pathologic stresses, and generate functional and anatomical responses to maintain homeostasis. An integration among neural, cellular, immune, and tear film related responses is the basis of its ability to react and adapt quickly. Proper function of the tear film, lids and conjunctiva and their neural, hormonal, immune connections are all essential in maintenance of the cornea (Rolando and Zierhut, 2001). The inability to adapt or failure of one or more of its components gives rise to vicious cycles of inflammation and damage, which if not promptly contained will initiate and maintain ocular surface disease. Ocular surface disease is therefore the result of a progressive cascade of events involving simultaneously or sequentially, one or more of the different components of the system that is unable to compensate, respond and heal (Aragona and Rolando, 2013; Baudouin et al., 2013).

The avascular, squamous corneal epithelium is innervated by a dense mesh of sensory processes derived from the ophthalmic branch of the trigeminal nerve (Muller et al., 2003). These nerve endings are responsible for nociception, cold and pressure sensation. The 7000 nerve receptors per square mm of the cornea and their fibres are involved in protecting the cornea from damage by modulating the blink response, stimulating the production of tears and maintaining the cornea in a healthy state through the production of trophic factors (You et al., 2000). Nerve malfunction is the hallmark of neurotrophic keratopathy (NK). Corneal epithelial cells constantly turnover by cell desquamation at the surface, cell regeneration at the limbus and centripetal sliding and migration from the limbus. When the epithelium is injured, cells surrounding the wound migrate onto the wound bed to re-establish cover. This repair requires a controlled and collaborative system of communication between epithelial and neuronal cells to facilitate re-synthesis of the damaged matrix, cell migration and restoration of architecture (Zieske and Gipson, 1986). If epithelial healing is impaired, the exposed stroma becomes vulnerable to enzymatic degradation, melting and eventually perforation; features that represent severe NK (Fini et al., 1998).

2. Brief History of Neurotrophic Keratopathy

That transection of the trigeminal nerve can lead to degenerative changes in the cornea was first described by Magendie in 1824 (Magendie, 1824), and Middlemore in 1835 recognized that the cornea was one of the most sensitive structures in the body (Middlemore, 1835). Changes of a destructive nature were not confined to the cornea alone; similar changes were noted in the skin corresponding to the area supplied by the nerve transected or damaged. The skin changes

included a sensory deficit, vasodilation, swelling and the development of trophic ulcers also described in the past as "sluggish" ulcers (Dubler, 1884). The term 'trophic' relates to nutrition. A trophic nerve would be one that is associated with nutrition or regulates the metabolism of cells. It was initially considered that the Vth cranial nerve carried trophic fibres (sympathetic) but this has never been proven. Besides the trophic hypothesis, various other hypotheses were put forth to explain the corneal changes that follow trigeminal nerve damage: irritative nerve action wherein the damaged corneal nerves generate harmful stimuli; vascular or vasomotor disturbance related to loss of control of limbal and conjunctival vessels; trauma, which is of real concern as wounds heal slowly and an eye that is protected from trauma (eye closure) suffers less; desiccation and dehydration; infection; consequential cell damage and abnormal cell metabolism. Of these, the hypothesis of altered cell metabolism is the most popular and most likely. This implies that there is a "lack of normal peripheral antidromic activity of sensory nerves" (normally an impulse travels from the site of origin in the soma along the axon towards the central neuron – orthodromic. Movement in the opposite direction is antidromic). This leads to the accumulation of metabolites which in turn cause tissue swelling, loss of vitality and desquamation with the formation of a trophic ulcer. The term used to describe this condition was neuroparalytic keratitis (Klein, 1943).

3. Nomenclature

Various terms are used to describe corneal nerve related pathology. These are listed in table 1. The nomenclature is based on the major clinical presentation of an epithelial defect (non-healing, slow-healing or persistent) and the underlying nerve pathology, which has a common acronym of 'NK' for neuroparalytic, neuropathic or neurotrophic keratitis/keratopathy. The

terms are used interchangeably and have the potential to cause confusion. The paper titled 'Neurotrophic Keratitis' from the Cambridge Ophthalmological symposium (Bonini et al., 2003) begins with the word 'Neurotrophic keratopathy' highlighting the inconsistency and the lack of a definitive term being assigned to the condition. 'Keratitis' is probably not the correct term to use even though inflammation is often associated with the pathogenesis or clinical presentation of the condition. The term 'Neurotrophic Keratopathy' with the acronym (NK) would be more appropriate as it encompasses the underlying nerve problem, the trophic effect on the cornea of nerve disease, both hypoaesthesia and hyper-excitable states, and does not place emphasis on 'inflammation', which is not the primary driver of the condition. The term 'Persistent epithelial defect' (or its variations) though a good clinical descriptor, can be associated with a variety of non-nerve related conditions. Hence it is more appropriate as a clinical sign than the name of a disease. We propose that 'Neurotrophic keratopathy' be used as the definitive term to describe the condition.

4. Definition

The definition of Neurotrophic keratopathy (NK) has remained consistent over the last couple of decades. At the Cambridge Ophthalmological Symposium in 2003 (Bonini et al., 2003), the following definition was used: "Neurotrophic keratopathy (NK) is a degenerative corneal disease induced by an impairment of trigeminal nerve. Impairment or loss of corneal sensory innervation is responsible for corneal epithelial defects, ulcer, and perforation." The American Academy of Ophthalmology published the following definition in 2008: "Neurotrophic keratopathy (NK) is a degenerative disease of the corneal epithelium resulting

from impaired corneal innervation. A reduction in corneal sensitivity or complete corneal anesthesia is the hallmark of this disease and is responsible for producing epithelial keratopathy, ulceration and perforation (Wells and Michelson, 2008).

Two recent definitions published on line by very reputable entities are presented below. The first one by Eye Wiki, a product of the American Academy of Ophthalmology, states that "Neurotrophic Keratitis (NK) is a corneal degenerative disease characterized by a reduction or absence of corneal sensitivity. In NK, corneal innervation by the trigeminal nerve is impaired" (Rabiolo and Woodward, 2017)." The other published on the Medscape is similar though more elaborate "Neurotrophic keratopathy (NK) is a degenerative disease characterized by decreased corneal sensitivity and poor corneal healing. This disorder leaves the cornea susceptible to injury and decreases reflex tearing. Epithelial breakdown can lead to ulceration, infection, melting, and perforation secondary to poor healing" (Graham, 2016).

All definitions of NK describe it as a "degenerative disease" and all include "impaired corneal innervation" as the underlying pathology. The emphasis is on "sensory nerves or corneal sensation". Recent studies have demonstrated the role of sympathetic innervation in corneal pathology (Yun et al., 2016), and the occurrence of corneal nerve hyper or aberrant regeneration, which is quite distinct from attrition or extinction of nerves in the sub-basal plexus (Al-Aqaba et al., 2011b; Al-Aqaba et al., 2012). The role of nerve sprouting and regeneration, though not resembling normal anatomy, is unclear but difficult to ignore. In dry eye disease nerve loss followed by nerve regeneration consequent to therapy has been described (Benitez del Castillo et al., 2004; Iaccheri et al., 2017; Tuisku et al., 2008; Zhang et al., 2005). When regeneration is a component of the pathophysiology, inclusion of the term 'degenerative disease' in the definition may introduce a contradiction. Moreover, the underlying conditions can have inflammation,

trauma, congenital anomalies and others as the predominant manifestation. It has also been shown that partial or total sensory loss is compatible with relatively healthy corneal epithelium (Dhillon et al., 2016), though NK when clinically manifest is associated with corneal hypoaesthesia or anaesthesia. With the above points in mind we propose the following definition: "*Neurotrophic keratopathy is a disease related to alterations in corneal nerves leading to impairment in sensory and trophic function with consequent breakdown of the corneal epithelium, affecting health and integrity of the tear film, epithelium and stroma*". Clinically this implies that NK is the likely diagnosis in the presence of an epithelial defect that does not heal or heals and breaks down repeatedly (changing shape and size of epithelial defect) in the presence of reduced or altered corneal trophic function and sensitivity.

5. Anatomy and Physiology of Corneal Nerves

5.1 Anatomy

The cornea is arguably the most sensitive structure in the human body. It is 100 times more sensitive than the conjunctiva (Wells and Michelson, 2008), 40 times more than dental pulp and over 400 times more than the skin (Bonini et al., 2003).

Corneal innervation is predominantly sensory, from the ophthalmic division of the Trigeminal (V cranial) nerve (Fig. 1). 1.5% (200 to 450 neurons depending on species) of the trigeminal ganglion neurons serve the cornea (Felipe et al., 1999; Launay et al., 2015; Marfurt et al., 1989). Each neuron can support hundreds to thousands of nerve endings in the cornea (LaVail et al., 1993; Marfurt et al., 1989; Morgan et al., 1978). The nasociliary branch of the

ophthalmic division of the trigeminal nerve enters the orbit through the superior orbital fissure and is the main nerve covering the ocular surface. Two or three long ciliary nerves and a communicating branch to the ciliary ganglion arise from the nasociliary nerve before it terminates as the infra-trochlear and nasal branches (other branches are the anterior and posterior ethmoidal nerves). Six short ciliary nerves arise from the ciliary ganglion and together with the long ciliary nerves enter the suprachoroidal space by penetrating the sclera around the optic nerve. They pass anteriorly, supply the iris and ciliary body and terminate in the peri-corneal (limbal) plexus. The limbal plexus thus has both sensory and autonomic nerves and is predominantly vasomotor in function (Marfurt et al., 2010).

A mixture of sensory and autonomic nerves pass through the limbus and enter the cornea in the middle third of the stroma in a series of large, radially-oriented nerve bundles and run forward and anteriorly in a radial fashion toward the central area, giving rise to branches that innervate the anterior and mid-stroma. The posterior stroma seems to lack innervation though some investigators have noticed a sparse innervation of the corneal endothelium (Leon-Feliu et al., 1978; ten Tusscher et al., 1988; Wolter, 1957). There is a loose sub Bowman's plexus of nerves from where fibres penetrate the Bowman's zone, predominantly in the mid periphery of the cornea and emerge in the sub-basal (epithelium) plane where they end in single or multiple bulb-like structures which probably represent the termination and folding of the nerve sheath (Al-Aqaba et al., 2010). From this point numerous neurites emerge and spread across the surface of the cornea, in the sub-basal plane, dividing dichotomously and re-anastomosing to form the sub-basal plexus. The neurites are generally oriented such that they converge to an area between the upper two thirds and the lower one third where they form a distinct whorl pattern (Al-Aqaba et al., 2010; Patel and McGhee, 2009). Terminal branches from the sub-basal plexus pass

anteriorly into the epithelial cell layers, terminating within or in between epithelial cells (Stepp et al., 2017). A small population of axons terminates in the stroma, while others form a close anatomical relationship with stromal keratocytes and macrophages (Muller et al., 1996; Seyed-Razavi et al., 2014).

Autonomic innervation consists essentially of sympathetic nerves from the superior cervical ganglion (SCG). The SCG is located close to the internal carotid artery at the level of the 2^{nd} and 3^{rd} cervical vertebrae. It receives preganglionic fibres from neurons located at the level of the 1^{st} and 2^{nd} thoracic spinal nerves. Postganglionic (postsynaptic) fibres from the SCG ascend in the carotid plexus around the internal carotid artery. Fibres destined for the eye leave the carotid plexus in the cavernous sinus and enter the orbit through the superior orbital fissure as the sympathetic root of the ciliary ganglion. Some fibres directly merge with the long ciliary nerves and others pass through the ciliary ganglion, without synapse, to emerge in the short ciliary nerves. Primates including humans have little sympathetic nerve supply to the cornea (Ehinger, 1971; Sugiura and Yamaga, 1968; Toivanen et al., 1987) compared to rabbits and cats where they constitute approximately 15% of the total corneal innervation (Marfurt and Ellis, 1993). Rat and cat corneas also receive parasympathetic fibres from the ciliary ganglion (Marfurt et al., 1998; Morgan et al., 1987; Tervo et al., 1979). However, this kind of innervation has not been confirmed in humans. All corneal sensory nerves derive from finely myelinated (A-δ) and unmyelinated (C) axons determined by the size and presence of myelin sheaths in the axon (Felipe et al., 1999). In the human cornea, central stromal axons are unmyelinated and run in the anterior stroma as large bundles parallel to collagen bundles. Most of the axons in these bundles are about 0.5 μm in diameter. However, few may be as large as 2.5 μm (Muller et al., 1996; Muller et al., 1997). On the other hand, more than 70% of the axons in rabbit corneas are

unmyelinated (Beuerman et al., 1983). The rest are finely myelinated axons that lose their myelin sheath within 1 mm after penetrating the cornea (Lim and Ruskell, 1978; Rozsa and Beuerman, 1982; Zander and Weddell, 1951). Myelinated axons are present in the central cornea in some mammals (Rodger, 1950; Whitear, 1960). As soon as they enter the corneal stroma, the nerve bundles lose their perineurium and continue as elongated structures running between the collagen lamellae.

5.2 Physiology

The physiology of corneal nerves is complex. Aspects relevant to NK are described and included herein. Electrophysiological examinations have revealed the existence of different functional types of ocular sensory neurons, including polymodal nociceptor neurons, cold thermoreceptor neurons, and selective mechano-nociceptor neurons (Belmonte et al., 2004a; Belmonte et al., 2004b).

The majority of the sensory nerve fibres innervating the cornea are polymodal nociceptors which are activated with near-noxious or noxious mechanical energy, heat, chemical irritants, endogenous chemical mediators released by damaged corneal tissue, and by inflammatory cells (Belmonte et al., 1991; Belmonte and Giraldez, 1981; Gallar et al., 1993; MacIver and Tanelian, 1993). When the stimulus causes tissue injury severe enough to trigger local inflammation, their threshold for activation decreases, and the impulse discharge evoked by suprathreshold stimulation increases. So called “sensitization” develops and may be associated with allodynia (pain evoked by innocuous stimuli), hyperalgesia (enhanced pain in response to noxious stimuli) and spontaneous pain (Stapleton et al., 2013). Reflex tear secretion caused by corneal stimulation seems to be chiefly due to activation of corneal polymodal nociceptors

(Acosta, Peral, 2004). The transient receptor potential (TRP) cation channels subfamily V member 1 (TRPV1) plays an important role in sensory transduction in polymodal nociceptors. It is expressed in intraepithelial nerve terminal endings in the corneal epithelium (Alamri et al., 2015; Guo et al., 1999; Murata and Masuko, 2006) and is activated by capsaicin, low pH, noxious heat and hyperosmolarity (Caterina et al., 1997; Davis et al., 2000; Straub, 2014). Acid-sensing ion channels (ASICs) and TRP cation channel subfamily A member 1 (TRPA1) also appear to contribute to chemical sensitivity of corneal polymodal nociceptors (Bandell et al., 2004; Bautista et al., 2013; Callejo et al., 2015).

The neuropeptides contained in some polymodal receptors (substance P and calcitonin gen-related peptide – CGRP) maintain corneal homeostasis and integrity by promoting corneal epithelial cell proliferation, migration, adhesion and differentiation (Garcia-Hirschfeld et al., 1994; Reid et al., 1993; Tran et al., 2000). Corneal epithelial cells in turn, release soluble factors (e.g. NGF and GDNF) that promote neurite extension and survival (Chan and Haschke, 1981; Lambiase et al., 2000). Furthermore, the lacrimal gland provides growth factors and nutrients and in turn, its function is influenced by sensory nerves. About 20-30% of peripheral axons innervating the cornea are selective mechano-nociceptors, which respond only to mechanical stimuli at an order of magnitude close to that required for corneal epithelial damage (Belmonte et al., 1991; MacIver and Tanelian, 1993). These mechano-nociceptors are probably responsible for the acute, sharp pain sensation induced by touching or scratching the corneal surface. Piezo2, a newly identified mechanically sensitive ion channel is present in about 30% of corneal sensory neurons in the trigeminal ganglion, but has not been described yet in the intraepithelial nerve terminals (Alamri et al., 2015; Bron et al., 2014). Cold thermoreceptors represent 10-15% of the total population of corneal sensory neurons (Belmonte et al., 2017). They change their activity

with both cooling and heating as well as with changes in osmolarity (Carr et al., 2003; Gallar et al., 1993; Parra et al., 2014; Quallo et al., 2015). Their activity is modulated by inflammation, which reduces their impulse activity as well as by peripheral injury that increases firing frequency (Acosta et al., 2013). The TRP subfamily member M8 is a cation channel that is important for cold sensation. It is activated by cooling, menthol, and osmolality values greater than 340 mOsm. (McKemy et al., 2002; Parra et al., 2010; Peier et al., 2002; Quallo et al., 2015). In the rabbit cornea, specific populations of c-fibers exist which are stimulated by acetylcholine possibly acting via a neuronal nicotinic receptor (Tanelian, 1991).

Nerve growth factor (NGF), epidermal growth factor (EGF), glial derived neurotrophic factor (GDNF) as well as brain derived neurotrophic factor (BDNF) are the main agents of an epithelial-nerve cross talk which plays a fundamental role in corneal wellbeing and healing (Muller et al., 2003). NGF, GDNF, their receptors TrkA and GFRa-1, as well as BDNF may also play an important role in maintaining corneal epithelial stem cells in the limbus (Qi et al., 2007). In addition, NGF seems to facilitate innervation of perivascular nerves to regulate blood flow in corneal neovascularization (Matsuyama et al., 2017). Recent evidence indicates, that significant and complex interactions exist between the nervous and immune system. Primary sensory neurons seem to be involved in maintaining the cornea's immune privilege (Belmonte et al., 2017). Moreover, peptidergic polymodal nociceptor terminals with their sensory neuropeptides substance P and CGRP contribute to the inflammatory response following tissue injury (neurogenic inflammation) (Belmonte et al., 2004a): CGRP has immunosuppressive effects, while substance P acts as a potent pro-inflammatory neuropeptide (Micera et al., 2006; Reynier-Rebuffel et al., 1994). Fractalkine (FKN, CX3XL1), produced by primary sensory neurons, plays an important role in the maintenance of corneal well-being, and disturbances in FKN/CX3CR1-

signalling may also result in corneal inflammation (Clark, 2014). NGF is a constitutive molecule present and produced in normal human corneas and important for the development and maintenance of peripheral sensory neurons. NGF and/or NGF-receptors TrkA and p75NTR are expressed in many corneal tissues including epithelium, endothelium, keratocytes and nerves, as well as by bone marrow (BM) derived cells present in the cornea (Lambiase et al., 2000; Sarkar et al., 2013). Tear NGF is increased after both photorefractive keratectomy and laser in situ keratomileusis (Lee et al., 2005). In addition, some semaphorins such as Sema7A and VEGF-A act as neurotrophic factors in the cornea and are able to influence inflammatory events (Li et al., 2011; Namavari et al., 2012; Takamatsu and Kumanogoh, 2012). T-cell-dependent inflammation involving IL-17, neutrophils, platelets, and VEGF-A seems to promote corneal nerve regeneration (Li et al., 2011; Namavari et al., 2012; Takamatsu and Kumanogoh, 2012).

In a healthy eye, bidirectional communication between nerves and the immune system forms a negative feedback loop that keeps both systems in check (Belmonte et al., 2017). Minor insults to the ocular surface are rapidly healed within a continuous trophic environment maintained by corneal innervation and the tear film (Mathers, 2000; Stern et al., 1998).

6. Incidence and prevalence

To determine the prevalence and incidence of a disease it is necessary to first agree upon a definition (DEWS report 2007). With regard to NK, as highlighted above, the definition has not changed for more than 15 years. In the context of the prevailing definition, NK has been classified as a rare/orphan disease (ORPHA137596) affecting 5 individuals or fewer in 10,000.

NK requires a fresh and joined approach by all stakeholders to enable effective treatment in the context of all options available.

There is a paucity of information in the literature regarding the prevalence and incidence of NK. The best evidence available (Sacchetti and Lambiase, 2014) is based on extrapolation from the two most common conditions associated with NK, which are herpetic keratitis (incidence of 1.22/10,000) and post-surgical nerve damage (incidence of 0.02/10,000); as being below 1.6/10,000. More specifically, NK develops in an average of 6% of herpetic keratitis cases, which has a prevalence of 149/100,000 (Labetoulle et al., 2005) and in 12.8% of herpes zoster keratitis cases, which has a prevalence of 26/100,000 (Dworkin et al., 2007). Other than herpetic keratitis, the most common cause of NK is neurosurgical intervention to treat trigeminal neuralgia that damages the trigeminal nerve (post-surgical incidence of 2.8%), which has a prevalence of 1.5/10,000, and an estimated prevalence of NK after neurosurgical procedure of 0.02/10,000 (Bhatti and Patel, 2005). Unfortunately, no epidemiological data can be found in the literature for a number of other common conditions known to cause NK such as chemical burns, diabetes, contact lenses and less frequent causes such as space occupying intracranial masses, multiple sclerosis and leprosy.

Geerling et al. (unpublished observations, 2017) in a retrospective case series from a subspecialist corneal clinic in Germany identified 38 eyes of 35 patients (17 males and 18 females with a mean age of 67 years) with NK over a two year period (2015-2016). They searched for patients who were treated with autologous serum eye drops, amniotic membrane transplantation, emergency corneal grafting or a combination thereof (commonly employed treatments for moderate to severe NK). 40.6% of emergency corneal grafts (13 out of 32), 15%

of amniotic membrane transplantations (28 out of 187) and 17.9% of autologous serum treatments (7 out of 39) were related to NK.

Although dry eyes are a feature of NK, dry eye disease and NK are different clinical entities. Some convergence is seen however, when laser refractive surgery is considered as a cause of neuropathic dry eye (Chao et al., 2014). The incidence of neuropathic dry eye following laser in-situ keratomileusis is estimated to be between 2 to 5% of Caucasian patients, rising to around 28% in Asians (Albietz et al., 2005; Azuma et al., 2014).

7. Aetiology and Pathogenesis of Neurotrophic Keratopathy

7.1 Etiology of Neurotrophic Keratopathy

Any persistent alteration of the corneal sensory innervation interfering with the function of the post-ganglionic fibres can cause NK (Sacchetti and Lambiase, 2014). (Fibres projecting from cortical/spinal nuclei to the trigeminal ganglion are pre-ganglionic and those projecting from the ganglion to the ocular surface are post-ganglionic). The common causes of severe NK are corneal herpes infections, ocular surface thermal and chemical burns, contact lens misuse and cranial neurosurgery. A number of events and ocular and systemic conditions can chronically affect the functioning of corneal nerves inducing NK. Unpublished observations [Figueiredo G, Baylis O, Lako M, Figueiredo FC] from a prospective phase II clinical trial in the UK on treatment of unilateral ocular surface burns related total limbal stem cell deficiency (LSCD) with *ex vivo* expanded autologous limbal stem cell transplantation demonstrated that that all 23 patients studied also had NK in the affected eye. The mean age of patients was 44.7 years (range 24-81, SD 14.19). The mean Cochet-Bonnet aesthesiometry measurement was 9.13 (range

0-30, SD 9.73) in the LSCD/NK eyes and 59.13 (range 50-60, SD 2.881) in the fellow normal eyes. The difference between the LSCD/NK and fellow eyes was statistically significant ($p<0.0001$, Wilcoxon rank test). A summary of the common causes of ocular surface nerve damage is given in table 2.

7.2 Pathogenesis of NK

In animal models, ocular nerve injury causes swelling of the squamous epithelial cells, loss of cell surface microvilli, abnormal basement production, acceleration of sloughing and loss of cells in to the tears, with epithelial thinning and breakdown (Mishima, 1957; Alper, 1975; Beuerman and Schimmelpfennig, 1980). The number of mitoses is diminished and as a consequence, epithelial healing is impaired leading to recurrent erosions and ulcerations (Bonini et al., 2003). Denervation of the cornea limits the extent and increases the duration of wound closure (Wilson and Ambrosio, 2001). In a model of NK created by surgical amputation of the trigeminal ganglion in albino rabbits, Akari et al demonstrated a delayed rate of healing, fewer desmosomes and excessive exfoliation of epithelial cells leading to persistent epithelial defects (Araki et al.,1994).

Many theories have been proposed to explain the pathogenesis of NK, including, desiccation of the corneal surface due to diminished lacrimal secretions as tear secretion is nerve stimuli dependent (Belmonte and Gallar, 2011); impaired corneal sensitivity leading to diminished protective blink reflexes, abnormal epithelial cell metabolism with subsequent failure to resist the effects of trauma, drying, and infection; and the loss of trophic influences provided

by corneal nerve fibres (De Haas, 1962; Duke-Elder and Leigh, 1965; Heigle and Pflugfelder, 1996; Paton, 1926; Wilson and Ambrosio, 2001). In most cases it is probably a combination of these factors, with a neurotrophic deficit playing a major role.

The reduced secretion of tear fluid and the loss of vitality of the epithelial cells can cause a reduction in the presence of neurotrophins on the ocular surface, in particular NGF, which is a neurotrophin essential to the development and survival of sympathetic and sensory neurons, and for trophic support after neuronal injuries (Sacchetti and Lambiase, 2017). It is normally present in the healthy cornea, where it regulates the proliferation and differentiation of epithelial cells. NGF also appears to be involved in epithelial and stromal interactions that induce stromal healing and remodelling. A reduced availability of NGF will result in impaired nerve and cornea functions (Chen et al., 2014; Di et al., 2017; Park et al., 2016). Reduced mitosis would lead to a slowing of or a dysfunction in the centripetal movement of cells from the limbus, thus affecting the cells in the centre of the cornea. As the cells here age, their ability to hold the tear film on the surface also reduces. This combined with the effect of the altered quality and amount of tear fluid may reduce the ability of the tear film to cushion the shearing stress of lid movements. The central cornea would then be most vulnerable and this can explain the central location of epitheliopathy at onset of NK and the subsequent ulcer. Reduced tear production is a major cause of decreased tear clearance with consequent accumulation of toxic agents and pro-inflammatory cytokines on the ocular surface, which may also be a contributing factor.

Excessive and rapid evaporation of tears from the ocular surface could be a source of epithelial keratopathy in dry eyes. Rapid evaporation causes a drop in corneal temperature,

which in turn will trigger acute and repeated stimulation of cold nerve sensors at the corneal surface. Over time, continuous stimuli will change the thermal sensor function into nociceptor (pain) sensor (Belmonte et al., 2009). Stimulation of peripheral nociceptors leads to the release of a variety of substances that can further stimulate the nociceptors and evoke release of pro-inflammatory mediators such as Substance P, CGRP, Neurokinin A (NKA), and Endothelin-3 (ET-3), and induce neurogenic inflammation.

Anatomical studies have shown a direct apposition of nerve terminals with dendritic cells, which are cells of the innate immune system. Neuropeptides released from nociceptors can induce degranulation or cytokine production in these cells. CGRP-containing nerve fibres are intimately associated with Langherans cells (LC) in human epidermis and CGRP is found at the surface of some LC. In three functional assays CGRP inhibited LC antigen presentation. These findings indicate that CGRP may have immunomodulatory effects in vivo and suggest a locus of interaction between the nervous system and immunological function (Hosoi et al., 1993).

Infrequent blinking associated with NK can thus induce and sustain an inflammatory environment and perpetuate epithelial keratopathy. Furthermore, there is some evidence suggesting that healthy epithelial cells of the cornea may work as Schwann cells on the local denuded nerve fibres (Stepp et al., 2017). Loss of epithelial support will make nerves vulnerable to damage and improper function building a vicious cycle of evaporation - excessive stimulation - neurogenic inflammation – epithelial damage - nerve hyper/ improper activity – inflammation. Evaporation from the tear film is not balanced by corresponding increase in tear secretion thus leading to a hyperosmotic environment, which induces cell apoptosis and inflammation with an increased expression of matrix metalloproteinases (MMP) initiating a cascade of events which perpetuate and worsen the condition (Baudouin et al., 2013). These collagenolytic enzymes

(especially MMP-2 and -9) are produced by corneal epithelial and stromal cells themselves (Geerling et al., 1999). An imbalance between the activators and inhibitors of MMPs is the main driver for progression and chronicity of the stromal ulceration and collagen melting, which can eventually lead to corneal perforation and loss of vision (Fini et al., 1992). Corneal epithelial damage also impairs its ability to maintain a difference in electric potential between the outer and inner surfaces. Loss of the electro negative repulsive charge favours bacterial adherence. This combined with the loss of a whole host of antimicrobial peptides (Mohammed et al., 2017) that are normally present in the tear film and actively secreted by the epithelium, favours microbial invasion and infection. Infection in turn augments stromal melting.

The impact of preservatives on the ocular surface, especially benzalkonium chloride (BAK) used in topical eye medications, deserves special mention. BAK is a tensioactive and cytotoxic compound widely used as preservative in ophthalmic solutions. It is known to induce pro-inflammatory and pro-apoptotic effects proportional to its concentration and is responsible for multiple effects on the ocular surface, specifically the induction of dry eye and chronic inflammatory changes (Baudouin et al., 2010). BAK has also been shown to be neurotoxic (Sarkar et al., 2012), causing corneal hypoesthesia and nerve damage (Labbe et al., 2012; Martone et al., 2009). Moreover BAK may severely affect corneal wound healing, delaying corneal epithelial wound closure in animal models, even below the concentration (0.01%), found in most ophthalmic solutions (Kossendrup et al., 1985; Nagai et al., 2010; Sharma et al., 2011). *In vivo* and *in vitro* models showed the negative impact of BAK-containing solutions on damaged corneas (Liang et al., 2012). These models offer easy and reliable investigations of the effect of substances on the wound healing process. *In vitro* wound-healing assays using corneal cell monolayers involve the making of a standardized ‘scratch’ in the monolayer with a sterile

439 micropipette tip under an inverted microscope (Fig. 2). Cell proliferation as well as rate and
440 extent of wound closure are easily followed and documented, allowing reliable investigations
441 and comparisons on drug toxicity profiles. Although most active compounds used in glaucoma
442 like beta-blockers or prostaglandin analogs are found to be almost neutral (Liang et al., 2012),
443 BAK reliably shows concentration-dependent delay in wound healing, with concentrations as
444 low as 0.001% being toxic and delaying wound closure. These models however do not take into
445 account the additional effects of BAK on tear film, goblet cells, corneal nerves and inflammatory
446 cells (Baudouin et al., 2010). Any non-healing corneal epithelial defect in an eye receiving
447 potentially toxic compounds like preservatives, antibiotics, steroids or non-steroidal anti-
448 inflammatory drugs should be considered as a possible iatrogenic consequence of therapy and
449 cessation of medication should be considered as a first step before embarking on elaborate
450 management regimes (Gomes et al., 2017).

451 The role of the conjunctiva in the pathogenesis of NK is not clear even though it
452 undergoes significant changes during the disease process including a decrease of goblet cell
453 density and loss of cell-surface microplicae. Gilbard and Rossi (Gilbard and Rossi, 1990)
454 demonstrated that although the conjunctival changes in NK were consistent with increase in tear
455 film osmolarity and surface changes as seen in dry eye disease, the corneal changes observed
456 with denervation, including slit-lamp findings, morphologic changes and decreases in glycogen,
457 were too severe and rapid in onset to be accounted for by osmolarity alone.

458 With greater degree of hypoaesthesia and anaesthesia, the patient has almost no
459 symptoms. Medical attention is delayed until the disease progresses to a stage where vision is
460 affected. However, the hypoaesthesia may affect only the denuded area (ulcer) with sensations
461 persisting in the surrounding cornea. Paradoxically, NK can present with symptoms of

neuropathic corneal pain as seen after Herpes zoster ophthlamicus. There is likely to be a spectrum from increased sensitivity and decreased threshold (hyperaesthesia) to absence of corneal sensations, with the former leading to the latter as the disease progresses.

NK related to herpes related keratitis is an enigma. Recurrent HSK results in persistent epithelial defects in the cornea ipsilateral to the affected trigeminal pathway. This is associated with hyperosmolarity, reduced tear break up time, reduced tear secretion (Schirmer I test) and reduced sensations not only in the affected eye but also in the unaffected eye (Rousseau et al., 2015; M'Garrech et al., 2013; Jabbarvand et al., 2015)**.** Unilateral herpes simplex and herpes zoster keratitis is associated with bilateral loss of corneal nerve receptors and dendritic cell infiltration (Hamrah et al., 2010; Hamrah et al., 2013; Cavalcanti et al., 2018) .The findings are mirrored in studies on rabbits with unilateral trigeminal axotomy, wherein bilateral loss of corneal nerves and immune cell infiltration was demonstrated (Yamaguchi et al., 2016; Yamaguchi et al., 2013). This suggests that unilateral affection of the (central) trigeminal pathways could trigger bilateral responses probably via neurogenic inflammation. In humans, though clinical manifestations are predominantly unilateral it is possible that subclinical NK is present in the other eye as well, in some cases. Whether amelioration of signs and symptoms by treatment of the affected eye leads to resolution of changes in the other eye remains to be seen.

7.3 Natural History of NK

When cornea sensitivity is impaired or lost, a sequence of events is triggered at the ocular surface leading to NK and its consequences (Fig. 3).

a. Tear secretion is reduced or abolished, the tear film is thinner and unstable. Mucin

484 distribution over the ocular surface is altered and irregular. The composition of tears
485 changes with regard to growth factors, cytokines, antimicrobial peptides and ions
486 adversely affecting epithelial homeostasis. Its physical ability to protect against lid
487 shearing forces is diminished.

488 b. Due to lack of trophic neuro-mediators, epithelial cells mitosis and maturation at the
489 limbus and centripetal migration are slowed down. This results in the accumulation of
490 older, less vital, pre-exfoliating cells in the centre of the cornea with poor ability to be
491 wetted, which can be easily damaged by friction with the lids during blinks.
492 Tear film changes and lack of trophic support may lead to epithelial irregularity and
493 grayish appearance which may affect the quality of vision.

494 c. Continuous epithelial damage results in chronic epithelial instability, with loss of
495 effective tight junctions and zonulae occludens, and increased risk of bacterial adhesion
496 and infection.

497 d. With time, the tear film alteration, epithelial pathology and reduced ability to provide
498 new cells, all combine to worsen the epithelial damage leading to an epithelial defect
499 characterized by its central position and by the presence of rolled borders made of cells
500 that are unable to adhere to the basement membrane possibly covered by denatured
501 mucins or bacteria, which are no longer cleared by adequate tear production.

502 e. Surface dryness, inflammatory cytokines and local epithelial damage leaves the
503 underlying stroma open to the destructive activity of MMP resulting in stromal melts
504 (Pflugfelder et al., 2005).

505 f. The extent and progression of stromal damage is determined by the balance between
506 activators and inhibitors of the proteases. Attempts at healing lead to unsuitable

collagen deposition, with irregular scar formation, loss of transparency and visual function.

g. Unchecked disease and ongoing metalloproteinase activity leads to perforation (Chotikavanich et al., 2009).

h. Eventually functional and anatomical loss of the eye occurs.

8. Clinical presentation and classification

Patients with NK will present in the early stage of the disease with symptoms of dryness, photophobia, and inability to read for a prolonged period of time due to epithelial changes and instability (see pathogenesis), impaired quality of vision and reduced blink. Symptoms are usually worse in the morning or in the presence of aggravating factors such as air conditioning, air travel, draught of hot air from car heating or prolonged use of some computers (draught of hot air from computer fans and reduced blinking associated with mental concentration) (Dua et al., 2014). Paradoxically, with worsening or severe disease, the symptoms of pain and discomfort may be less or absent due to sensory dullness related to hypoaesthesia or anaesthesia of the cornea. Symptoms of visual impairment appear when the central cornea is significantly involved. Clinical signs relate to those associated with the underlying condition and those due NK, usually a combination of the two.

8.1 Signs related to NK

Early signs are similar to those of dry eye with rapid tear-break up time, narrow tear meniscus and inferior one third conjunctival and corneal punctate staining with fluorescein (superficial punctate

keratitis SPK). The blink rate can be reduced and irregular (normal 17 blinks per minute) (Bentivoglio et al., 1997). The cornea reflex (lustre) becomes dull and the epithelium appears cloudy with epithelial irregularities. In NK related to laser refractive surgery (Ocular surface syndrome) (Alio et al., 2007; Ambrosio et al., 2008), SPK is centrally located and can wax and wane (Fig. 4 a-c). With increasing severity, the epithelial erosions coalesce and become larger (coarse erosions) and a frank epithelial defect, usually centrally located, appears (Fig. 4 d-f). Attempts at healing of the defect are slow and often incomplete. The area of the defect changes as the epithelium heals and breaks down repeatedly. Eventually the defect becomes permanent (persistent epithelial defect, PED) with smooth or rolled and opaque edges. The epithelium around the perimeter of the defect is loosely attached to the underlying Bowman's layer as evidenced by the sub-epithelial seepage of fluorescein dye, beyond the edge of the defect. The exposed stroma is vulnerable to the effect of proteases resulting in melting, that can lead to perforation (Fig. 4 g-i). The ulcer is usually sterile however secondary microbial infection can occur which can lead to rapid progression of melting and perforation. (Fig. 5). Stromal involvement can also manifest as edema, striae and Descemet's folds. Cells in the anterior chamber can be seen and frank hypopyon should be carefully evaluated and followed since it can be sterile or a sign of secondary infection. In vivo confocal microscopy examination, in the presence of frank corneal hypoaesthesia, can show an intact sub-basal plexus (pre-ganglionic affection of the Vth nerve) with a relatively better prognosis (Dhillon et al., 2016). In the majority of cases however, the sub-basal plexus is deficient.

Corneal vascularization is variable in its occurrence and severity. NK itself can cause stromal vascularization but wide variations related to the underlying cause, can be seen. Some infections like with acanthamoeba cause little vascularization whilst herpes virus infections cause the most (Faraj et al., 2016). Ocular surface inflammation maybe obvious as conjunctival hyperemia or could be completely absent.

8.2 Signs related to underlying disease

555

556 Signs related to the underlying cause could manifest as lagophthalmos or reduced blink
557 reflex, signs of previous herpetic keratitis with scarring and vascularization or patches of iris
558 atrophy, lattice or granular dystrophy, enlarged beaded corneal nerves, scarring from previous
559 corneal infections, limbal stem cell deficiency, advanced diabetic retinopathy or pan-retinal
560 photocoagulation. Optic disc swelling or atrophy may suggest orbital or cranial lesions. Reduced
561 or absent sensation in the dermatomes supplied by the trigeminal nerve which may be associated
562 with other cranial nerve affection may give clues to the underlying cause. Seventh cranial nerve
563 palsy may affect the prognosis of the disease from corneal exposure due to lagophthalmos and
564 reduced blink reflex and can be modified by the presence or absence of a good Bell's
565 phenomenon.

566

567 *8.3 Classification*

568 Traditionally NK has been classified into 3 stages as described by Mackie (Mackie,
569 1995).

570 Stage 1 of neurotrophic keratopathy demonstrates the following:

571 • Rose Bengal staining of the inferior palpebral conjunctiva (lissamine green is now the
572 standard dye used instead of rose Bengal)
573 • Decreased tear breakup time
574 • Increased mucous viscosity
575 • Punctate corneal epithelial fluorescein staining

576 Stage 2 is characterised by:

577 • Epithelial defect - Usually oval and in the superior cornea
578 • Defect surrounded by a rim of loose epithelium
579 • Edges may become smooth and rolled
580 • Stromal swelling with folds in the Descemet's membrane
581 • Sometimes associated with anterior chamber inflammatory activity

Stage 3 is characterised by:

- Stromal lysis/melting
- May result in perforation

Dryness and visual aberrations are the main symptoms of NK and reduced or absent corneal sensations, the main and arguably the pathognomonic clinical sign. However, with current understanding of corneal nerve pathology using in vivo confocal microscopy and post-mortem whole mount staining of corneal pathology it is evident that aberrant re-generation and hyper-regeneration of nerves also occurs, which could by inference lead to corneal hyper-aesthesia and account for the occurrence of symptoms that are out of proportion to the clinical signs (Al-Aqaba et al., 2011b; Al-Aqaba et al., 2012; Wolter, 1964, 1966). This however is usually seen in some cases in early disease and current available methods of evaluation do not allow the assessment of increased sensitivity. Reduced sensitivity and the consequences thereof constitute the classical manifestation of NK. Direct imaging of nerves has enabled a degree of quantification based on nerve density, tortuosity, thickness, reflectivity and aberrations such as looping, coiling, irregularity in diameter, presence of 'growth cones' and truncation (dead ends). It has also been demonstrated that nerve anomalies can be localised to some parts of the cornea whilst others can have normal physical appearance of nerves and their distribution. It is not clear whether altered sensitivity is restricted to the areas of nerve anomalies or more generally reflected in the cornea. Nevertheless, it is good practice to test sensations in the centre and in the peripheral four quadrants. Patient symptoms appear to be more generalised, with no specific corneal/ocular surface localisation, regardless of the location of nerve anomaly.

Although the Mackie classification has been in vogue for a number of years, we propose the following adaptation, which would be more clinically relevant and indicate severity and prognosis. Examples are given in figure four.

- Mild [Epithelial changes only without epithelial defect]: Epithelial irregularity without frank epithelial defect; tear film instability and symptoms (hyper-aesthesia) with reduced or absent sensations in one or more quadrants of the cornea.
- Moderate [Epithelial defect without stromal defect]: Frank persistent epithelial defectand corneal hypo-aesthesia/anaesthesia.
- Severe [Stromal involvement]: Stromal involvement from corneal ulcer to lysis to perforation, with corneal hypo-aesthesia/anaesthesia.

Epithelial disturbance, frank non-healing epithelial defect and stromal lysis usually but not necessarily follow sequentially (Figs. 6-8) . All other clinical signs of NK are variable and do not appear or progress sequentially as is often determined by the underlying condition for example in a case of chemical burn the patient may present with severe NK without having progressed through NK of mild and moderate severity. It is important to understand the difference between an abrasion and an ulcer as both are technically ‘epithelial defects’. Abrasion implies the rubbing or scraping away of cells from the surface of an area of the cornea, skin or mucous membrane; whereas an ulcer is a breach of the continuity of the epithelium of any of the above mentioned tissues, due to sloughing related to inflammation and tissue necrosis. Abraisons generally heal rapidly while ulcers take longer to heal, festering as ‘non-healing epithelial defects’.

9. Diagnosis and Differential diagnosis

Diagnosis of NK is based on the clinical interpretation of the history, general examination of the patient, slit lamp examination of the eye and findings of some diagnostic tests. Clinical examination and tests are directed towards features of NK and of any possible underlying condition.

9.1 Ocular symptoms

Symptoms of NK are elaborated in the section on clinical features and should be specifically explored as the presenting symptoms of NK can vary according to the severity of the disease. Other symptoms such as dryness, photophobia, lacrimation and visual disturbance or impairment can be present and should be explored and documented. There is lack of correlation between symptoms and signs. Neuropathic corneal pain can be a presenting feature and severe corneal signs can be present with disproportionally minimal pain.

9.2 Clinical History

Patients often have a history of features related to an underlying condition (table 2). Previous hospital visits for ophthalmic and non-ophthalmic consultations, previous ocular or brain surgery, ocular or head trauma, use of topical medication (specifically preserved eye drops) and systemic medication (eg. neuroleptics and antipsychotic drugs). Systemic chronic conditions such as diabetes and multiple sclerosis can be present. Topical anaesthetic misuse is often missed unless suspected in individuals with specific professions (eg. welders, metal workers).

9.3 Examination

9.3.1 Neurological Examination

This includes assessing cranial nerve function, which may help to localize trigeminal damage. Concurrent abnormalities of the third cranial nerve with sixth cranial nerve may indicate damage in the cavernous sinus or localize an intracranial aneurysm. Pupillary abnormalities may indicate the status of third nerve, as well as defects, in the sympathetic innervation of the iris. The presence of an afferent papillary defect in association with corneal hypoesthesia would localize the lesion to the intra-conal orbit. Pupil reactions consistent with Adie's pupil have also been associated with alterations in corneal sensation.

Abnormalities of the 7th & 8th cranial nerve may indicate damage from acoustic neuroma or neuro-surgery. Damage to 7th nerve may can lead to exposure of the ocular surface due to lagophthalmos, which will worsen prognosis of patients with NK especially in the absence of Bell's phenomena.

9.3.2 Ophthalmic Examination

9.3.2.1 External exam (eyelids and conjunctiva)

Eyelid function is critical to the prognosis of neurotrophic keratitis and progression of advanced disease. Lid features to note are ectropion, entropion, misdirected lashes or ptosis (oculomotor nerve damage or mild ptosis in the presence of corneal infection). Lid scarring may be present secondary to removal of periocular infiltrative tumors or chemical or thermal burns. The conjunctivae in NK patients generally show a lack of conjunctival injection i.e. 'white' eye. The presence of 'red eye', however, would indicate the presence of co-existing inflammation usually related to secondary infection. Subconjunctival fibrosis may be present, which could be associated with chronic autoimmune disease and/or severe dry eye.

669 *9.3.2.2 Slit lamp examination*

670 Examination of the cornea in NK with the slit lamp may reveal a spectrum of changes, as
671 described in the section on 'Clinical features'. Other corneal changes which may indicate
672 previous infections or recurrent corneal ulceration should be noted, such as the presence of
673 vascularization and/or scarring. Keratitis related Herpes virus infection is the commonest cause
674 of corneal vascularization (Faraj et al., 2016). Anterior chamber examination may reveal flare,
675 keratic precipitates, cells or frank hypopyon from active anterior uveitis, which may result from
676 non-viral corneal infection or inflammatory reaction from herpetic kerato-uveitis. Iris
677 examination may show sectoral transillumination indicating iris atrophy secondary to herpes
678 kerato-uveitis/iris pigment epithelialitis.

679 *9.4 Vital Staining*

680 Fluorescein and lissamine green dyes are useful in assessing subtle changes in the
681 epithelium (mild NK) and frank epithelial defects (moderate and severe NK). As tear film
682 anomalies are integral to the pathophysiology of NK, tear assessment is important. Besides other
683 tests such Schirmer's test, both these vital dyes are useful in assessing 'dry eye' signs such as
684 tear meniscus height, tear breakup time and punctate corneal and conjunctival erosions.
685 Assessment of tear film osmolarity is considered to be important in dry eye disease but its role in
686 NK is not clear (Belmonte et al., 2017). Ocular fundus examination may reveal diabetic
687 retinopathy, optic nerve pallor (multiple sclerosis) or swelling from an intracranial neoplasm.
688 The above account illustrates that, as with any ocular assessment, with NK too, it has to be
689 thorough and complete and not restricted to the cornea or ocular surface.

690 *9.5 Diagnostic tests*

9.5.1 Corneal Sensation

Assessment of corneal sensation is fundamental to the diagnosis of NK. The algorithm for diagnosis is given in the diagram (Fig. 9). Clinically, corneal sensation is assessed by using a 'wisp' of cotton applied to both corneas (Fig. 10a). Patient's reaction is noted and compared between the eyes. NK patients typically show reduced blinking/sensation to the stimulus. Corneal sensation is reduced or normal, if it is normal then NK unlikely. Some authors have stated that an absence of the nasal-lacrimal tearing reflex along with ipsilateral loss of sensation in the nasal mucosa presents a high risk for subsequent neurotrophic corneal ulceration, so there may be a basis for testing this.

Corneal sensation can be (semi)quantitively measured by the Cochet-Bonnet aesthesiometer or the Belmonte non-contact gas aesthesiometer (BNGA) (Fig. 10b and 10c). The former is a contact instrument and the latter is not. With the Cochet-Bonnet aesthesiometer, corneal sensitivity is assessed observing the patient's subjective reaction to different lengths of a protruding nylon filament applied to the cornea which is extended from 60mm to 5mm with a corresponding change in force from 11 to 200 grams/mm. After touching the cornea in the quadrant to be tested, pressure is applied on the filament to induce a gentle bend. At this point the patient should appreciate the touch of the filament tip. The length is reduced in 5mm steps until the patient appreciates the touch. The longer the length at which the patient feels the touch of the filament, the higher the corneal sensitivity. Measurements are performed in each quadrant of the cornea and data recorded accordingly (Golebiowski et al., 2011).

The BNGA works by stimulating the cornea with a calibrated gas emission from an injector kept close to the cornea and subsequent blink response. The BNGA is mounted on a slit-lamp (similar to an applanation tonometer) with the gas injection tip kept perpendicular to the

cornea, the subject is instructed to look at a fixation target at 3 m, and the injection tip is kept 5mm away from the surface, (the distance is measured with a transparent ruler). Subjects are instructed to close and open their eyes just before triggering the stimulus. By varying the flow, temperature and composition of gas (CO2 concentration) this device can assess different components of corneal sensation i.e. mechanical, chemical and thermal sensitivity. The technique has been found to be safe and reproducible and its 'non-contact' nature makes it safer than contact methods (Belmonte et al., 1999; Teson et al., 2012).

9.5.2 Imaging Corneal Nerves

In vivo confocal microscopy (IVCM) (Fig. 11) allows qualitative and quantitative assessment of corneal nerves in health and disease. Nerve density, tortuosity, angulation, thickness and reflectivity are assessed using image analysis programmes. IVCM has been used to detect corneal nerves changes in a variety of conditions such as keratconus, bullous keratopathy, diabetic neuropathy and herpes simplex keratitis (Al-Aqaba et al., 2011a; Al-Aqaba et al., 2011b; Cottrell et al., 2014; Messmer et al., 2010). In diabetic neuropathy, analysis of corneal nerve density and morphology has demonstrated a correlation between reduction in fibre density/branching and severity of somatic neuropathy. The method is sensitive enough to detect significant structural abnormality in the corneal nerves of patients deemed to have mild diabetic neuropathy by conventional tests and can help detect onset of diabetic peripheral neuropathy prior to clinical manifestation. In pre-ganglionic (trigeminal ganglion) lesions and partial ganglion lesions, corneal sensitivity can be absent or diminished but IVCM demonstrates a normal sub-basal plexus with mild NK. In post-ganglionic or complete ganglionic lesions the sub-basal plexus is attenuated or lost, corneal sensations are reduced and the risk of developing moderate to severe NK is high (Dhillon et al., 2016). Accurate imaging and quantitative analysis

of images of sub-basal and stromal nerves can be affected in corneas presenting with severe NK due to the possible influence of concurrent stromal oedema, infiltration, melting or scarring.

9.5.3 Anterior Segment Optical Coherence Tomography (ASOCT)

Fourier domain ASOCT can demonstrate corneal nerve abnormalities (radial keratoneuritis) such as during active acanthamoeba keratitis (Yamazaki et al., 2014) and can be used to assess treatment response. However, current instrumentation has insufficient resolution to resolve corneal nerve architecture changes where the sole abnormality is loss of corneal sensation. ASOCT comes in handy to assess corneal thickness changes as may occur during moderate to severe NK; providing both morphometric and qualitative data. ASOCT can be used for measuring the depth of stromal ulcerations and stromal thickness changes occurring over time in NK (Fig. 12), facilitating the diagnosis of cases at risk of perforation and improving the follow-up analysis after surgical tectonic grafts of amnion or corneal transplantation (Nubile et al., 2011).

9.6 Differential diagnosis

Several chronic diseases can lead to NK and others can mimic NK, the key distinction being the alteration/absence of corneal sensation. The terms primary and secondary NK have been used as descriptors but there are no differences clinically in the corneal manifestations once the process is driven by loss of trophic and sensory function. Systemic causes such as trigeminal nerve damage from tumour, trauma or surgery, or neuropathy associated with diabetes and multiple sclerosis can be considered as examples of primary NK whereas damage to the corneal innervation from direct insult to the cornea such as following viral infections, corneal transplantation or refractive surgery, can be considered as examples of secondary NK. Central

and peripheral NK are term that also reflect the site of lesions that can cause NK, analogous to primary and seconday NK. In central NK corneal and conjunctival sensations are likely to be affected compared to peripheral NK where only corneal sensations are likely to be predominantly affected. In the majority of cases NK is a unilateral disease. Where the cause is diabetic neuropathy or multiple sclerosis it can be bilateral.

Dry eye disease, contact lens related disorders, blepharo-keratoconjunctivitis, limbal stem cell deficiency, exposure keratopathy, radiation keratopathy, topical drug and preservative toxicity and chronic eye rubbing are important conditions that can have overlapping features with NK and may remain as independent entities until corneal sensitivity is affected.

10. Management

Left untreated, NK can evolve into a devastating condition culminating in anatomical loss of the eye. Short of this, loss of vision is common even with treatment. Management of NK can be divided into medical management, non-surgical intervention and surgical management. These can be considered in a step-ladder approach according to NK stage/severity but are not exclusive as often a combination of options may need to be considered. The objective of treatment is to arrest progression and reverse NK changes that have occurred at the time of presentation. In mild NK (stage 1), the objective is to prevent epithelial breakdown and encourage healing of epithelial erosions. In moderate NK (stage 2), the objective is to encourage re-epithelialisation of the denuded stroma and prevent progression to stromal melting. In severe NK (stage 3), the objective is to prevent perforation and promote healing. Throughout the course of NK maintenance of comfort and optimizing vision are also therapeutic considerations.

10.1 Medical Management of NK

Considerations for the medical management depend upon the severity / stage of NK and pathology of the underlying disease process. Therapeutic approaches are broadly divided into strategic areas that encompass categories of treating the underlying disease process, treating any concurrent infections, preventing disease progression, promoting epithelialisation, providing tear replacement, reducing inflammation, preventing stromal tissue loss or perforation and avoiding complications. The current step-ladder of interventions for NK according to severity is summarised in table 3**.**

10.1.1 Treatment of concurrent inflammation

10.1.1.1 Infection

Culture or PCR of forniceal swabs to identify common and unusual pathogens that may be present in abundance on a compromised ocular surface driving inflammation through activation of innate immune responses, is essential (Kugadas and Gadjeva, 2016). Identified organisms (bacteria, fungi, and viruses) should be treated. However, when infection is suspected but cultures are negative, empirical treatment with Azithromycin 1g orally for 3 days is advised. For persistent epithelial defects, secondary infection delaying healing should be excluded and the eye treated with broad spectrum topical antibiotics. Wherever possible, toxic aminoglycosides such as gentamicin should be avoided unless sensitivities dictate otherwise. Topically administered quinolones can also be toxic to the ocular surface (Ayaki et al., 2012; Mencucci et al., 2011; Walter and Tyler, 2001). Drug toxicity should be suspected when initial clinical improvement changes to clinical worsening and increased inflammation. The ‘up-down’ test,

where in the upper bulbar conjunctiva appear white compared to the lower injected bulbar and fornicial conjunctiva, is a useful early indicator of drug toxicity. (Dua et al., 2012).

10.1.1.2 Minimise ocular irritants and conservative treatment

Awareness of iatrogenic causes of ocular toxicity is critical (Dart, 2003). All preserved therapy should, wherever possible, be discontinued. Detection of corneal deposits such as fluoroquinolone crystals (ciprofloxacin, ofloxacin) (Claerhout et al., 2003; Mitra et al., 2007), or hydroxyapatite formation due to a combined effect of hyaluronates and phosphates (Bernauer et al., 2006a), or calcific deposits after the use of steroid-phosphate (Schlotzer-Schrehardt et al., 1999), retinoic acid (Avisar et al., 1988), or lubricant phosphates (Bernauer et al., 2006b) should lead to clinical approaches that minimise potentially damaging drug/chemical precipitation reactions on the ocular surface.

Patients should also be advised to refrain from using irritating peri-ocular cosmetics, increase humidity by wearing protective moisture chamber wrap-around glasses or goggles, and consider increasing dietary omega-3 fatty acids (Eicosapentaenoic acid (EPA)) or linoleic acid and gamma-linolenic acid (Flaxseed oil). Fish oils have been shown to provide modest benefit to the ocular surface by inhibiting pro-inflammatory mediators (Prostaglandin E2, Leukotriene B4, IL-1 and TNFα) (Kangari et al., 2013).

10.1.1.3 Reduce Inflammation

The use of non-preserved topical medications is essential to minimise ocular surface inflammation. Meibomian gland dysfunction should be treated with warm compresses possibly with the aid of proprietary eye-lid warming devices, lid massage and hygiene with diluted sodium bicarbonate or commercially available lid hygiene wipes. The use of matrix

metalloproteinases inhibitors in the form of low dose tetracyclines or macrolides is advised. The mainstay is with the use of topical non-preserved glucocorticoids such as prednisolone or dexamethasone (Geerling et al., 2011). Delayed wound healing related to steroid medication, pharmacokinetically only occurs at higher doses or with longer duration of treatment. Nevertheless, inhibition of stromal healing, may increase the risk of corneal stromal melting and perforation, thus their use should be considered with caution. Vigilant screening for steroid-related raised intraocular pressure and optic neuropathy is required. Soft steroids such as medroxyprogesterone 1%-2% and androgens (if available) may ameliorate this risk. Topical non-steroid anti-inflammatory drugs (NSAID) treatment does not improve the healing process. NSAIDs are generally avoided due to their epithelial toxicity and the risk of corneal ulceration (Guidera et al., 2001; Gaynes and Fiscella, 2002; Lee and Himmel, 2006; Feiz et al., 2009).

Topical ciclosporin is only licensed for use in primary or secondary dry eye disease. Ciclosporin 0.1% (Ikervis® SDU) is licensed for severe keratitis of dry eye disease not responding to ocular lubricants. In the US and Far East, Ciclosporin 0.05% (Restasis®) may be a useful alternative. PADciclo 0.06% is currently undergoing clinical trials. The veterinary preparation, Ciclosporin 0.2%, (unlicensed, Optimmune®) may also be considered if an ointment with lubricating properties is required. Although tacrolimus 0.03% to 0.1% has been shown to be effective in combating ocular surface inflammation, its use in the context of NK has not been studied (Fukushima et al., 2014; Kiiski et al., 2014).

10.1.2 Tear substitution

Ocular lubricants reduce biomechanical shear forces and dilute pro-inflammatory mediators in the tear film thereby promoting epithelialisation. They are generally classified

according to viscosity ranging from low to high and are summarised in table 4. Due to the toxicity associated with preservatives (Baudouin et al., 2010; Geerling et al., 2001; Gomes et al., 2017), unpreserved lubricants are prescribed, many of which can be bought over the counter by the patient. Carmellose agents are cytoprotective (Garrett et al., 2007), and hyaluronate ligation to CD44 expressed on injured ocular surface epithelial cells, deliver anti-inflammatory properties in addition to facilitating epithelial wound healing (Gomes et al., 2004). Other compounds such as guar gums, liposomes and soybean/mineral oil combinations, help stabilise the phospholipid layer whilst other agents confer osmoprotection (glycerine, L-Carnitine, erythritol, threalose). In patients with filamentary keratitis, mucolytics (acetylcysteine 5-10%) may be beneficial. Recent clinical trials indicate promising results for the use of diquafosol 3% (a P2Y2 receptor activator that improves mucociliary clearance and mucin production) and rebamipide 2% (that stimulates transmembrane mucin MUC16 biosynthesis) in multifactorial dry eye disease, although the exact benefit for NK is yet to be determined. Alternative non-preserved lubricants such as saline 0.9% (that has no excipients) or balance salt solution should be considered in resistant cases.

Nutritional tear substitutes (serum eye drops) are considered when all treatment options have been exhausted. Unlike other pharmaceutical tear supplements, nutritional substitutes contain substances that are also present in natural lacrimal tears and support ocular surface epithelial growth and regeneration. These include growth factors (epidermal growth factor and transforming growth factor beta), Vitamins (A, C), glucose, natural antimicrobials (surface IgA, defensins, lysozyme), and proteins involved in wound healing (fibronectin) (Rauz and Saw, 2010). Most commonly used are autologous serum eye drops (Azari and Rapuano, 2015; Semeraro et al., 2014; Turkoglu et al., 2014), but there is a risk of instilling circulating antibodies or pro-inflammatory mediators in patients with systemic diseases e.g. Multiple sclerosis, mucous

membrane pemphigoid that have the potential to exacerbate disease processes in the eye. Allogeneic serum eye drops (available in the UK, Germany, New Zealand) are obtained from young healthy male donors and avoid cyclical oestrogen hormone variances that may have pro-inflammatory effects on the surface of the eye. Human umbilical cord serum has been shown to contain higher levels of growth factors, including nerve growth factor, and other constituents similar to those in tears, and has been used to treat a variety of ocular surface conditions including NK (Yoon et al., 2005; Yoon et al., 2007). The response to cord blood serum appears to be related to the severity of NK with mild to moderate (stages 1 and 2) lesions responding quicker than severe (stage 3) defects. (Erdem et al., 2014). However, small volumes obtained from the placenta, limits general use. Platelet-rich plasma (PRP) is abundant in growth factors that promote ocular surface regeneration (Hartwig et al., 2004; Hartwig et al., 2005). Despite the differences in methods used to prepare this product for clinical use, studies indicate that PRP gives better results than autologous serum and can lead to healing of persistent epithelial defects where autologous serum drops have failed. They also have a good safety profile (Kim et al., 2012; López-Plandolit et al., 2010; Soni and Jeng, 2016). Its content of biologically active proteins, growth factors, and biomaterial scaffolds make PRP a therapeutic agent promoting ocular surface wound healing and regeneration (Anitua et al., 2016). PRP has also demonstrated efficacy, as a monotherapy, in the management of post laser refractive surgery ‘ocular surface syndrome’ (Alio et al., 2017).

10.1.3 Prevent stromal tissue loss

Stromal degradation occurs after the release of proteolytic enzymes from recruited inflammatory cells, activation of clotting and kinin cascades that induce further inflammation and stromal loss. Matrix metalloproteinases inhibitors (oral tetracyclines and topical acetylcysteine) restrict neutrophil collagenase and epithelial gelatinase gene expression, suppress alpha-1 antitrypsin degradation and scavenge reactive oxygen species (Ogut et al., 2016; Hahn et al., 2016; Abdul-Hussien et al., 2009; Sekundo et al., 2002). Ascorbate applied topically or given systemically, provides cofactors for collagen synthesis as well as scavenging for oxygen free radicals. Topical citrate, if available, is a calcium chelator inhibiting neutrophil degranulation and release of proteolytic enzymes as well as inhibiting collagenases (Parker et al., 1985).

10.1.4. Biological medical products

Drugs given systemically targeting mediators that fuel inflammation or delay wound healing have gathered momentum over the past two decades, although very few biologics have been licensed for ocular use. Topically administered biologics or biosimilars provide an attractive area for drug development. Murine NGF (Bonini et al., 2000; Lambiase et al., 1998), Substance P and Insulin-like growth factor (Yamada et al., 2008) were the first mediators to show encouraging results (Fig. 13). A number of novel treatment modalities have emerged through clinical trials and are available for clinical use. These include recombinant human NGF (rhNGF, cenegermin (betaNGF) (European Medicines Agency, 2017), lifitegrast 5% (lymphocyte function-associated antigen-1 (LFA-1) antagonist) (Perez et al., 2016), ReGeneraTing Agent [RGTA] – matrix therapy agent, Cacicol20, Thymosin beta 4, Conenzyme Q10, Substance P, Netrin-1 (class of proteins involved in axon guidance and cell migration) and

912 Nexagon® (an antisense oligonucleotide that downregulates expression of the gap junction
913 protein Cx43, which is increased in pathological conditions with persistent epithelial defects)
914 (Guerra et al., 2017). Many of these agents promote healing in a generic way by combating
915 inflammation (lifitegrast) or rejuvenating the stroma by providing binding sites for growth
916 factors to promote healing (RGTA). NGF specifically targets the deficit in NK by replacing the
917 nerve growth factor and promoting epithelial healing and nerve health.

918 Cenegermin is a recombinant form of human nerve growth factor (rhNGF) produced in
919 Escherichia coli as a pro-peptide, which is later cleaved to mature NGF. The molecule is
920 identical to human NGF (European Medicines Agency, 2017). The European Medicines Agency
921 recently (July 2017) granted Cenegermin 20 µg/ml (Oxervate®) full marketing authorization for
922 the treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) NK in adult
923 patients by, making it the first approved medical treatment for this specific indication. The
924 efficacy and safety of cenegermin were evaluated in two independent, multicentre, randomised,
925 double-masked, vehicle-controlled clinical studies (NGF0212 and NGF0214) in patients with
926 moderate or severe NK refractory to non-surgical treatments. In both studies patients received
927 cenegermin or vehicle 6 times daily in the affected eye(s) for 8 weeks, and were followed-up.

928 Study NGF0212 (NCT01756456) conducted in Europe, enrolled a total of 174 patients (mean
929 age 61±16 years); 156 patients were assessed independently for efficacy, comparing two
930 different dosages of the medicinal product with 20 and 10 µg/ml cenegermin to vehicle (52
931 patients per arm) (Mantelli et al., 2017; Sinigaglia and NGF Study Group, 2014). Study
932 NGF0214 (NCT02227147), run in US, enrolled 48 patients (mean age 65±14 years) treated with
933 cenegermin 20 µg/ml or vehicle (24 patients per arm) (Chao et al., 2017). Complete corneal
934 healing of the persistent epithelial defect or corneal ulcer (the key efficacy endpoint, defined as

the greatest diameter of corneal fluorescein staining <0.5 mm) after 4 and 8 weeks of treatment for patients who received cenegermin 20 µg/ml or vehicle was compared. There was a statistically significant improvement to complete healing of the cornea at 4 and 8 weeks of treatment (58.0% and 74.0% respectively) compared to vehicle (19.6% and 43.1% respectively) ($p < 0.001$ and < 0.002) in study NGF 0212. In study NGF 0214 the difference was statistically significant at 8 weeks of treatment (69.6% with cenegermin and 29.2% with vehicle, $p < 0.006$).

10.2 Non-surgical interventions

10.2.1 Eyelid closure

Tarsorrhaphy, the suturing of the eyelids to narrow the interpalpebral fissure is a cornerstone of NK management. Non-surgical techniques that can be used as an alternative to tarsorrhaphy include eyelid closure with tape, pressure patching, pad and bandage and botulinum toxin injection induced ptosis. In principle these have the same objective of covering the cornea, protection against the environment and effects of lid blinks. Contact lenses may also serve a similar purpose.

Placing an adhesive tape (transpore) across the closed eyelids is a simple intervention to keep the lids closed. The limitation of taping is that if repeated removal and (re) application is required to instill eye drops or examine the eye, the delicate skin of the lids can excoriate and be bruised. The ‘Apache patch’ and invention of Mr B Donaldson of Aberdeen, Scotland, is a clever eye patch device which has two components, a ‘T’ shaped part for the upper lid with adhesive backing to the horizontal limb and a small rectangular part, with adhesive backing, for the lower lid. The Velcro components can be separated and reattached without removing the device from

the lids. The Frost suture (can be considered a surgical intervention), which involves the placement of suture (4 ‘0 silk) horizontally through the skin of the upper (or lower) lid close to the lash line, serves a similar purpose. Use of an eye pad with tape or bandage provides complete closure with some pressure. Usually two pads are used, one folded in half and applied on the closed lids to fill the space of the anterior orbit, and the other placed on this and attached with a couple of strips of tape running from the forehead to cheek or held in place with an eye bandage. These are useful when closure for the whole day is required and not when the eye has to be opened frequently during the day. The drawback of needing to undo the patch or eyelid tape every time an eye drop is due has to be administered, makes them impractical. Importantly, care has to be taken to ensure that the lids do not open under the patch as this would allow the patch to rub against the cornea and aggravate the condition.

Botulinum toxin induced ptosis is an exception to the above. It provides a safe, rapid and effective means of closing the eye whilst still allowing for easy access to examine or instill eyedrops (Fig. 14). An injection of botulinum toxin is administered at the upper border of the superior tarsal plate or over the belly of the levator palperae superioris muscle and induces paralysis thereof causing the upper lid to droop and completely cover the cornea. The effect typically lasts 6 to 12 weeks. The number of units, dilution and volume to be injected varies according to form of botulinum toxin used. Another consideration is that the effect of the toxin is not immediate and can take several hours or a couple of days. Hence repeat injections should be avoided in the same week. In cases where only partial closure occurs, lagophthalmos may result in exposure and compound the problem and a further injection of botulinum toxin may be required (Kirkness et al., 1988; Schilimow and Wiechens, 2016).

10.2.2 Therapeutic Contact Lenses

Therapeutic contact lenses are fitted to maintain the integrity of the ocular surface tissues where improvement of vision is secondary benefit. Commonly used biomaterials are silicone hydrogels and rigid gas permeable, that are fitted as corneal, limbal, semi-scleral or scleral lenses. Rigid gas permeable scleral contact lenses are vaulted away from the cornea and supported by the anterior sclera. The design creates a reservoir between the lens and cornea that captures therapeutic substances and increases retention time of therapeutic agents and lubricants on the ocular surface. One such device is the prosthetic replacement of the ocular surface ecosystem (PROSE) device. The use of contact lenses in the context of NK must be accompanied by extreme vigilance. The reduction of sensation reduces 'alarm' signals for infection. If used, prophylactic use of topical non-preserved antibiotics is advised (Baenninger et al., 2014).

Innovative devices using a combination of amniotic membrane and a contact lens are in vogue. The OmniLenz® and Prokera™ (see later) are examples. OmniLenz® consists of a soft contact lens lined with a circular disc of vacuum dried amniotic membrane and is applied directly on the corneal surface. The amniotic membrane rapidly hydrates and provides a soft biological cover to the defect. It is suggested that the release of molecules from the amniotic membrane favour healing. Evidence from clinical trials is lacking though anecdotal experience with single cases has shown a beneficial effect.

10.2.3 Punctal Occlusion

Punctal occlusion increases the retention of natural tears enhancing the healing process (Cohen, 1999; Guzey et al., 2001). Punctal occlusion can be performed by various methods (Baxter and Laibson, 2004). Punctal plugs – these can be temporary or short acting collagen

plugs, or permanent punctal or canalicular plugs. The punctae can be also be permanently occluded with thermal cautery. Other methods of occluding the puncta, include argon laser, suturing of the punctum, or canalicular ligation (DeMartelaere et al., 2006; Hutnik and Probst, 1998; Liu and Sadhan, 2002).

Punctal plugs can be associated with complications, such as pyogenic granulomas, bacterial colonization, extrusion, and local irritation (Kim et al., 2005; Sugita et al., 2001; Tai et al., 2002). Timing is critical as early insertion during active inflammation may lead to the accumulation and stagnation of tears loaded with pro-inflammatory cytokines and pro-inflammatory mediators gene expression on the ocular surface (Tong et al., 2016).

10.3 Surgical intervention

Surgery is often required in advanced disease refractory to medical management, mainly in moderate and severe NK (stages 2 and 3). Medical and surgical options are not mutually exclusive and are often combined.

10.3.1 Permanent punctual occlusion

Where temporary punctual occlusion has been successful in improving the tear reservoir, epiphora has not occurred and there is no prospect of a return of normal lacrimation then permanent punctual occlusion may be considered. This is particularly beneficial in patient likely to require lifelong punctual occlusion in whom punctal plugs can give rise complications stated above. Cauterisation of the vertical part of the canaliculus and punctum with heat is the standard method to achieve this.

10.3.2 Tarsorrhaphy

Tarsorrhaphy provides a more definitive closure of the eyelids by approximating the upper and lower lids. This is considered as the gold standard in NK treatment in several centres (Fig. 15). Closure of the lids protects the cornea from the environment, prevents epithelial damage by the friction caused by eyelid movement especially when the lid margins are irregular and keratinized (Cosar et al., 2001), conserves tear fluid and provides a reservoir of tears to keep the eye constantly moist and theoretically, the approximation of the vascular palpebral conjunctiva to the corneal surface affords additional unknown benefit. Tarsorrhaphy can be temporary or permanent, depending largely on the natural history of the underlying etiological condition (Allen and Malinovsky, 2003), partial (lateral, medial, central) or complete, depending on the severity of NK.

Of the various techniques of performing a tarsorrhaphy, the most common is suturing the lids to each other over bolsters. This tarsorrhaphy reverses when the suture is removed and often the suture loosens after a few days or weeks. If the lid margins are denuded prior to suturing, the tarsorrhaphy is permanent, and if they are not, it is temporary. The advantage of (lateral) tarsorrhaphy over corneal patching is that the eye can be examined, the patient has vision, and the risk of infectious keratitis is reduced (Ali and Insler, 1986; Cosar et al., 2001; Panda et al., 1999). Tarsorrhaphy should be considered in all cases of persistent epithelial defects that fail to respond to medical treatment and/or non-surgical interventions (Tuli et al., 2007). If healing occurs, the tarsorrhaphy opening may be enlarged after a few weeks, but opening the tarsorrhaphy prematurely may results in a recurrence of corneal epithelial breakdown, especially if total corneal anaesthesia persists.

10.3.3 Debridement

At times, the leading edges of the healing epithelium may thicken and become rolled or

heaped, impeding migration across the defect. In such cases, the epithelium at the edges of the defect may be removed, effectively enlarging the defect. This triggers the healing response in the surrounding epithelium promoting migration to close the defect (Katzman and Jeng, 2014).

10.3.4 Amniotic Membrane Transplantation (AMT)

The amniotic membrane AM) is a versatile tissue that has caught the imagination of ophthalmologists and has been used for a wide variety of indications across several ophthalmic subspecialities. Its efficacy has been adequately demonstrated (Azuara-Blanco et al., 1999; Dua et al., 2004; Gomes et al., 2005), but equally it is at times used "as something to do rather than something that does" (*hsd*) (Clare et al., 2012; Joseph et al., 2001; Rahman et al., 2009). AM can be used as a graft (inlay) or a patch (onlay) (Bonini et al., 2003). When the AM is applied such that epithelium migrates on the membrane and the amnion becomes incorporated in the cornea, it is termed a 'graft' (Fig. 16). Conversely if the healing epithelium migrates under the AM and the amnion later falls off or is removed, it is termed a 'patch'. At times two membranes can be used one as a graft and the other as a patch over the graft. AMT can be combined with tarsorrhaphy but individually they have shown efficacy in the treatment of refractory neurotrophic corneal ulcers (Khokhar et al., 2005). Multiple pieces of AM cut to fit the shape of the defect can be stacked and finally covered by a graft or patch (Prabhasawat et al., 2001). The amnion allows keratocytes to migrate in the AM stroma and lay down collagen/scar tissue, which helps build the tissue at the site of melt. Multiple layers of amniotic membrane can integrate into the corneal stroma with resulting increase in corneal thickness; however keratocyte-mediated wound healing and remodeling of the incorporated amniotic tissue induces progressive contraction and changes in tissue transparency (Nubile et al., 2011). In the context of NK it has proven efficacy and is

usually used in severe NK (stage 3) but has been used in mild and moderate NK (stages 1 and 2). (Gris et al., 1999).

Fresh, cryopreserved (Amniograft), freeze dried (Ambio dry) and vacuum dried (Omnigen) amnion are available and all have demonstrated efficacy to a lesser or greater extent, with the latter two offering advantages of ease of storage and transportation at room temperature. All human derived tissue carries a serious risk of transmission of infectious disease. Though 'fresh' amnion is still used in some parts of the world, the practice does not allow sufficient time for a thorough testing for microbial contamination. Where serological testing is performed, the donor is tested at the time of donation and the tissue quarantined for 6 months, when a repeat test is performed. The material is released for clinical use only when both tests are negative. With PCR testing on tissue samples, it is possible to release tissue within a week, when theoretically it would be classed as 'fresh'. The membranes can be applied to the defect with tissue glue (fibrin glue, Tisseel®) or sutures. Lyophilized AM has been shown to have lower concentrations of proteins/growth factors (Rodriguez-Ares et al., 2009). AM has many features that make it extremely useful for the prevention and treatment of corneal ulceration (Tseng et al., 2004). Its basement membrane is composed of collagen types IV and VII, laminin 1 and 5 and fibronectin (Cooper et al., 2005). The laminin and fibronectin assist in epithelial cell adhesion and are therefore useful in treatment of PEDs (Cameron et al., 1988; Nakagawa et al., 1990). The stroma also contains multiple growth factors (EGF, TGF-α, KGF, HGF, bFGF, TGF-β1, -β2), anti-angiogenic factors (thrombospondin-1 and collagen VIII), TIMPs (1,2,3 and 4) and anti-inflammatory factors (IL-1 receptor inhibitor and IL-10) that may help in the resolution of ulcers and decrease scarring (Koizumi et al., 2000). The major use of AMT in corneal pathology is in the management of neurotrophic ulcers (severe NK) and PEDs. Kruse et al. evaluated multilayer

AMT in neurotrophic ulcers that had failed after at least 4 weeks of conventional therapy with lubrication, patching, or bandage contact lenses. They found that all the ulcers resolved at 4-5 weeks following AMT, but the surface layer of AMT disappeared faster than the ulcer healed, and multiple layers were necessary to achieve resolution of the ulcer (Kruse et al., 1999). Chen et al. performed a similar study on longstanding neurotrophic ulcers of various etiologies that had failed conventional therapy (Chen et al., 2000). They found that 76% of the eyes had rapid epithelial healing within 16 days. However, more than half of their patients also needed adjunctive therapy with tarsorrhaphy, bandage contact lens, or bandage amniotic membrane, reiterating that single layer AMT may not be sufficient for severe neurotrophic ulcers.

Prokera™, which consists of an amniotic membrane clipped into a dual PMMA ring set, has been used for the treatment of chronic ulcers. The advantage of this device is that it does not need sutures for placement and can be easily slipped into the eye like a large scleral contact lens (Suri et al., 2013). Other commercially available are Amnion (Bio-Tissue, Inc., Miami, FL), fresh frozen, and Ambiodry2 (IOP Ophthalmics, Costa Mesa, CA), freeze-dried. A suspension of homogenized amniotic membrane in BSS has been used topically in patients with ulcers refractory to conventional therapy. Healing of all ulcers occurred by 28 days following institution of therapy (Bonci et al., 2005).

10.3.5 Tissue adhesives

Tissue adhesives have been used for the closure of corneal defects and perforations for many years (Bloomfield et al., 1963; Refojo et al., 1968). In the presence of a small perforation (less than 3mm) the application of tissue adhesive on the lesion, followed by the application of a soft bandage contact lens or AMT is the procedure of choice. Larger defects require a conjunctival flap or lamellar keratoplasty (Mantelli et al., 2015). Two basic types of adhesives

are used in ophthalmology, synthetic (cyanoacrylate) and biologic (fibrin glue) (Bhatia, 2006). Cyanoacrylate polymerizes rapidly in the presence of water (tissue fluid) and also releases formaldehyde during polymerisation. Formaldehyde contributes to its antibacterial activity against most gram-positive organisms (de Almeida Manzano et al., 2006; Diaz-Valle et al., 2003). It forms a rigid, impermeable plaque on the surface of the eye, which needs to be covered with a bandage contact lens to offer protection and avoid pain and trauma to the upper lid. The contact lens also prevents the glue being dislodged by lid movement. It remains in the eye long-term, as it is not biodegradable. Multiple perforations or a single perforation that continues to leak from the edge of the first patch of glue application, requires one or more overlapping further applications (Fig. 17). When there is a perforation with iris prolapse, the double drape technique can be used. The prolapsed iris is covered with a circular disc of plastic drape without glue and this in turn is covered with a larger disc of plastic drape with cyanoacrylate glue. The second disc adheres to the corneal tissue around the first disc, which prevents the glue from directly adhering to the iris (Gandhewar et al., 2013). The epithelium usually grows under the glue and healing of the defect usually dislodges the glue.

Fibrin glue polymerizes relatively slowly and is therefore less effective in frank perforations with a brisk leak. Fibrin is also rapidly degraded, but the addition of aprotinin (antifibrinolytic agent) delays lysis for up to 10-14 days. Placement of a bandage contact lens after fibrin glue application also helps to retard degradation possibly by preventing access of polymorphonuclear cells and their proteases. The fibrin scaffold allows migration of keratocytes/fibroblasts and myofibroblasts, which in turn lay down collagen and help close the defect. Fibrin glue carries a theoretical risk of disease transmission as it is derived from pooled plasma. The advantage of fibrin glue is the higher comfort level and the absence of toxic

metabolites (Sharma et al., 2003).

10.3.6 Conjunctival Flap

A conjunctival flap may be indicated to prevent progression of the epithelial defect to perforation (Pushker et al., 2001). Total conjunctival flaps are more useful in patients with severe stromal damage and poor visual prognosis. Partial or bridge flaps may be used for small or peripheral ulcers. Conjunctival flaps have some disadvantages that include corneal perforation under the flap, flap retraction, poor reversibility and the need for an operating room. Despite the disadvantages, conjunctival flaps can be helpful because they halt the inflammatory process, and eliminate need for frequent instillation of medication.

The procedure of using a flap of bulbar conjunctiva to cover nonhealing corneal ulceration was first described by Trygve Gundersen in 1958 (Gundersen, 1958). Gundersen's flaps were frequently used for this purpose, but they fell into disfavor because they were difficult to fashion, especially in patients who had undergone multiple ocular surgeries, and they sometimes retracted. Other modalities for treating these conditions, such as disposable therapeutic contact lenses and AMT, were also much easier to use. However, in selected cases, conjunctival flaps may be superior, as they can replace an unhealthy stromal bed with healthy basal tissue on which epithelium can grow. In addition, they provide vessels and a blood supply to diseased cornea. These vessels aid in healing of resistant infections and provide serum-based growth factors. Vascularized structures are also very resistant to ulceration and perforation (Conn et al., 1980). The original flap procedure involved performing a 360º peritomy, debriding the corneal epithelium completely, and mobilizing the superior conjunctiva to cover the entire cornea. Various modifications have been made to that original technique in an attempt to decrease complications and increase the success rate, especially the use of partial pedicle grafts

(Alino et al., 1998; Khodadoust and Quinter, 2003; Sandinha et al., 2006). It is generally accepted that conjunctival flaps need to retain a pedicle to ensure continued supply of blood. This makes mobilization and advancement of conjunctiva on to the cornea difficult in cases where the underlying pathology for NK, such as chemical burns, affects the conjunctiva also. Dua et al. proposed use of a free autologous conjunctival graft from the opposite eye or from a distal surviving site in the same eye (Dua et al., 2012). The free graft covers the affected cornea in whole or part; the peripheral margin of the graft or at least part of it and the edge(s) sutured to viable tissue (with a blood supply intact). This allows blood vessels to connect with the vessels and in the graft that carry blood to the affected cornea, helping it heal. Other methods that have been tried for grafts to maintain globe integrity include buccal mucous membrane grafts, split thickness dermal grafts, and tenon's capsule grafts (Ma'luf and Awwad, 2005; Mauriello et al., 1988; Reim et al., 1992).

10.3.7 Corneal transplants

When all other options have failed to heal progressive corneal ulceration, a tectonic corneal transplant is often the last resort. Corneal transplants are preferably done as a planned procedure after the active ulceration has resolved and all inflammation has settled, though often it has to be performed in cases with impending perforation of perforated corneas. Risk of rejection and failure is greater with 'hot grafts' and grafts performed in background of NK are at high risk of failure (Jonas et al., 2001). However, tectonic grafts that are performed to preserve the structural integrity of the cornea have some advantages over other treatment modalities. The vision is often better than that achieved with glue, amniotic membranes, or conjunctival flaps if the visual axis is involved (Killingsworth et al., 1993; Vanathi et al., 2002). Corneal grafts may be lamellar or penetrating. The advantage of lamellar transplant is that the anterior chamber is

not entered in corneas that have not perforated. However, they are often technically challenging and may have poor visual outcomes (Soong et al., 2000). Jonas et al. compared the outcomes of penetrating keratoplasty in patients with corneal ulcers and patients with corneal scars from healed corneal ulcers (Jonas et al., 2001). They found that the visual outcomes were poorer and there were more episodes of rejection and loose sutures in the tectonic grafts. However, the visual outcomes were much better than with the lamellar transplants. Elective corneal transplantation for visual rehabilitation in patients with NK carries a high risk of failure. These patients have poor epithelial wound healing and are prone to inflammation. Both of these factors significantly increase the risk of melting, perforation and rejection.

A conjunctival flap is recommended when descemetocele or perforation recurs despite previous corneal transplantation (Vasseneix et al., 2006). The Boston keratoprosthesis implantation has emerged as an effective modality for visual rehabilitation in such patients (Katzman and Jeng, 2014; Pavan-Langston and Dohlman, 2008).

10.3.8 Direct neurotisation

Direct neurotization of the cornea using the contralateral supraorbital and supratrochlear branches of the ophthalmic division of the trigeminal nerve has been performed for restoring the corneal sensitivity in patients with unilateral facial palsy and anesthetic cornea. Terzis et al (Terzis et al., 2009) described a novel surgical procedure in which donor nerve branches are inserted at the contralateral anesthetic corneal limbus for sensory neurotization. Use of the sural nerve for this purpose has also been described (Bains et al., 2015; Elbaz et al., 2014). This surgical technique, although difficult to perform, preserves ocular anatomy and cosmesis and restores function. A step-ladder approach to the use of medical, non-surgical and surgical

interventions in the management of NK is given in table 3.

11. Future Directions

Testing corneal sensitivity is key to recognition and diagnosis of NK. Corneal sensitivity testing is not routinely undertaken in clinical practice and is inadequately performed with the help of a cotton wisp or tissue paper rolled to a fine tapering end with bare fingers (unsterile). This, at best gives a qualitative estimate of the central cornea as it is usually performed in the central cornea ignoring the four quadrants. The Cochet Bonnet aesthesiometer gives a reasonable quantitative estimate of corneal sensitivity. Its nylon thread is re-usable, usually cleaned with a sugical wipe but is not sterile. The Belmonte aesthesiometer is a non-contact device but for practical reasons is not widely used. As a result, NK remains an under-diagnosed condition. There is a strong need for a method or device that is clinically practical and easy to use in busy clinics, yet providing a quantitative (or semi quantitative) assesment.

Corneal hyperaesthesia is recognised as a feature of corneal pathology and may also be part of the process that evetually culminates in hypoaesthesia and complete loss of sensation.This can be associated with increased firing of existing nerves or abberant regeneration of nerves. There is no method available to test increased sensitivity. In future, as understanding of hyperaesthesia and methods of assessing it will become important.

The pathophysiology of NK is ill-understood and often compounded by the pathophysiology of the causative underlying condition or agent. This makes for inadequate staging or grading of the condition and treatment strategies are difficult to target towards specific factors in the etiopathogenesis. In addition, interactions between disease processes and iatrogenic interventions

make diagnosis and treatments even more difficult; therapeutic protocols lack standardization and duration of treatment remains unclear as reccurences may occur if treatment is tapered too early or in cases where the underlying condition cannot be cured. Nevertheless, affection of the sensory nerves of the cornea is common to all stages/grades of NK. Until recently there was no drug that addressed this factor. The advent of NGF with proven clinical efficacy through clinical trials offers a lot of promise. Other topically administered products that promote epithelial healing, are in the pipeline. These products should become available for clinical use in the near future leading to post marketing clinical trials, which in turn will establish the true potential of the products.

Surgical neurotisation of the cornea with nerve grafts is an elaborate procedure, still in its infancy but with exciting and promising possibilities. The foreseeable future will see improvement in surgical techniques and improved outcomes making the surgery available to more patients by a greater number of trained surgeons. High resolution in vivo confocal microscopy imaging of the cornea following neurotisation or treatment with rhNGF or Netrin-1should allow direct visualisation of the new nerves sprouting from the transplanted trunks.

Patients with NK manifest local (eye specific) symptoms, general health and social and psychological problems. Due to duration of the disease, its potential visual impact and the burden of the range of treatment options, quality of life can be severely affected by NK. There is a lack of a specific NK related tool to accurately assess quality of life (QoL) in these individuals. The main objectives of future therapies will thus be to tackle all these complex issues to heal the cornea, to prevent or reverse vision impairment and improve quality of life of NK patients.

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15. Legends for Figures

Fig. 1. Diagram illustrating the afferent sensory pathway from the cornea and conjunctiva to the trigeminal ganglion and the efferent sympathetic and parasympathetic nerve pathways. Adapted and modified from Stern 2013.

Fig. 2. Effect of benzalkonium chloride (BAK) on cultured corneal epithelial cells. H0 (hour zero) the vertical gap created by the 'scratch' in a confluent layer of corneal epithelial cells is visible. H8 (hour 8) the gap is fully closed to establish a confluent sheet again compared to the BAK group where the defect persists.

Fig. 3. Sequence of events leading to epithelial disturbance, epithelial loss and stromal lysis as neurotrophic keratopathy progresses through different stages/grades of severity.

Fig. 4. Grades/stages of neurotrophic keratopathy (NK). (a) Slit lamp broad beam illumination showing central, confluent punctate lesions giving the central cornea a dull lack-lustre appearance following photorefractive keratectomy (ocular surface syndrome) (OSN). (b) The same cornea stained with fluorescein showing that most of the lesions take up stain highlighting the epitheliopathy. (c) OSN following laser in-situ keratomileusis. Fluorescein stain of the cornea shows coarse, centrally located, almost confluent 'keratitis'. (a – c represent grade/stage 1 NK). (d) The central cornea shows a diffuse haze with a large epithelial defect. (e) The central cornea is hazy with an epithelial defect with superficial and deep stromal vessels. (f) There is a large corneal epithelial defect with a relatively clear stroma. Images d-f represent grade/stage 2 NK.

The white, rolled margins of the defects is a typical sign of a non-healing epithelial defect. (g) Slit view showing stromal melting to mid depth in a case of herpes zoster keratitis. (h) Deep stromal ulceration in a child with congenital anaesthetic cornea. (i) A large corneal ulcer in a case of treated acanthamoeba keratitis. Inset illustrated the stromal roughening, over which the epithelium did not migrate. Note the absence of corneal vascularization. Images g to i represent grade/stage 3 NK.

Fig. 5. Complications of neurotrophic keratopathy (NK). (a) Three months following a chemical burn the eye shows limbal ischaemia in the inferior half with a large hypopyon. (b) The same eye as in (a) stained with fluorescein. A large non healing epithelial defect is seen. Corneal sensation was absent in the lower half. (c) NK with superadded bacterial infection, stromal melting and ectasia. (d) Same eye as in (c) showing that the area of epithelial defect, stained with fluorescein, corresponds to the stromal involvement. (e) NK in a penetrating corneal graft in an aphakic eye, showing a ‘silent’ central perforation with prolapse of the vitreous. (f) NK with Descemetocele and perforation showing aqueous leakage (Sidel positive). The corneas in (e) and (f) developed perforation in the absence of infection.

Fig. 6. Right eye of a patient with mild/stage 1 neurotrophic keratopathy (NK). (a) The lower half of the cornea shows multiple, almost confluent, punctate erosions stained with fluorescein. The upper half has a normal tear film. Inset shows the white appearance of the epitheliopathy. Cochet-Bonnet aesthesiometery measured 60mm in the upper half and 15mm in the lower half. (b) Higher magnification broad beam slit lamp illumination shows the white lesion on the right

hand side of the beam and 'vesicular' appearance or intra-epithelial microcysts on the left side of the beam. Intraepithelial vesicular lesions are part of the epitheliopathy of NK. Superficial lesions rupture and stain with fluorescein.

Fig. 7. Left eye of the same patient as in figure 5. (a) The patient underwent penetrating keratoplasty for herpes simplex virus keratitis related scars. Post-operatively she presented with moderate/grade 2 neurotrophic keratopathy (NK). An epithelial defect with underlying and surrounding stromal haze is seen. (b) On fluorescein staining the ulcer is delineated. The surrounding epithelium shows coarse punctate keratitis. (c) NK progressed to severe grade/ stage 3. Various treatment options were tried including an amniotic membrane patch. The ulcer healed but left a scarred vascularized cornea, shown a year later. (d) Two years post-graft the corneal graft has failed with a dense scar and further vascularization. Corneal sensations were absent. The case illustrates that with conventional therapeutic interventions, 'successful' healing of severe NK can be associated with severely compromised vision.

Fig. 8. Progression of neurotropic keratopathy (NK). (a) Following successful treatment of acanthamoeba keratitis, there was marked reduction of corneal sensation (Cochet-Bonnet 5 mm) with moderate/stage 2 NK. (b) NK progressed with stromal lysis and (c) perforation, despite treatment.

Fig. 9. Diagnostic algorithm for neurotrophic keratopathy.

Fig. 10. Testing corneal sensation. (a) Testing corneal sensation with a wisp of cotton. This is a qualitative test and easy to perform at he bedside. However, the cotton is usually not sterile or even if so, is drawn into a this wisp with 'un-sterile' fingers. (b) testing corneal sensation with the Cochet-Bonnet aesthesiometer. The fine nylon thread is sterilised by wiping it with an alcohol swab. (c) The set up of the Belmonte aesthesiometer. A controlled jet of air is targeted on the cornea and the patient's response is both observed and interrogated.

Fig. 11. (a) A case of severe neurotrophic keratopathy (NK) examined by in-vivo confocal microscopy (IVCM). (b) IVCM of the ulcerated area appears as a dark hypo-reflective patch with scattered fine hyper-reflective speck (possible inflammatory cells/reactive keratocytes) and surrounded by the hyper-reflective epithelial cells of the ulcer margin. (c) A large stromal nerve is seen as a hyper-reflective line in the deep stroma of the peripheral non-ulcerated area. (d) The stroma in the vicinity of the ulcer shows disorganization with reactive keratocytes. (e) The epithelium in the vicinity of the ulcer shows altered morphology and absence of the sub-basal plexus.

Fig. 12. Anterior segment optical coherence tomography (ASOCT) in neurotrophic kertopathy (NK). (a) Severe NK with stromal melting. (b) ASOCT of the cornea illustrating the depth of the ulcer and its contour. The facet is filled with instilled tear drops. The density of the stroma (yellow red colour) is greater in the stroma around the ulcer. Anterior bowing of the posterior corneal layer can be appreciated indicating the start of a Descemetocele.

Fig. 13. Response to treatment with recombinant nerve growth factor (NGF) eye drops. (a) Moderate grade/stage 2 neurotrophic keratopathy. (b) & (c) complete closure of the epithelial defect occurred with NGF drops instilled six times a day for a few weeks.

Fig. 14. Beneficial effect of botulinum toxin induced ptosis in neurotrophic keratopathy (NK). (a) The cornea shows a large area of moderate grade/stage 2 NK. (b) Complete ptosis of the upper lid is achieved with botulinum toxin injection. (c) Healing of NK at 2 weeks and (d) complete healing at 4 weeks.

Fig. 15. Tarsorrhapy, arguably the most effective surgical intervention in neurotrophic keratopathy (NK). (a) A cornea with unilateral chemical burn was treated with autologous limbal transplant. A persistent epithelial defect is seen (NK moderate grade/stage 2). (b) A lateral tarsorrhaphy was performed. (c) Complete healing of NK is seen. (d), (e) and (f) are corresponding fluorescein stained images showing the original epithelial defect, reduction in size of the defect and complete healing respectively.

Fig. 16. Amniotic membrane transplant (AMT) in management of severe neurotrophic keratopathy (NK). (a) Severe (stage 3) NK following trabeculectomy operation, which did not respond to medical management. The central stroma is necrotic and surrounded by a gutter of stromal lysis. (b) The ‘gutter’ is epithelialized but no epithelium has grown over the central necrotic stroma (fluorescein stained). (c) An AMT (Omnigen 500 graft) with a running 10 ‘O nylon suture was used to cover the defect after dissecting off the necrotic stroma.

Fig. 17. Corneal perforation managed by application of cyanoacrylate tissue adhesive. (a) A case of bacterial corneal ulcer, medically treated with intensive antibiotics. The ulcer was rendered sterile but remained as a persistent defect with reduced sensation, progressing to perforation at two sites. Multiple patches of cyanoacrylate glue were required to seal the perforations. (c) Two months later, the glue has been dislodged revealing a scarred and vascularized cornea. The perforations have sealed and the anterior chamber is formed.

Table 1. Terms used to describe corneal nerves related pathology.

Non-Healing corneal epithelial defects
Persistent corneal epithelial defects
Slow-healing corneal epithelial defects
Neuropathic keratitis (epithelial defects)
Neurotrophic keratitis/keratopathy
Neuroparalytic keratitis/keratopathy
Ocular surface syndrome and Neurotrophic epitheliopathy post-Lasik

Table 2. Common causes of ocular surface nerve damage that may lead to neurotrophic keratopathy

- *Genetic* (Morishige et al., 2014)
  - Riley–Day syndrome (familial dysautonomia)
  - Goldenhar–Gorlin syndrome
  - Mobius syndrome
  - Familial corneal hypoaestesia
- *Systemic*
  - Diabetes mellitus. (Lockwood et al., 2006)
  - Leprosy
  - Vitamin A deficiency
  - Amyloidosis
  - Multiple sclerosis
- *Central nervous system*
  - Neoplasm
  - Aneurysms
  - Stroke
  - Degenerative disorders of the central nervous system (Alzheimers, Parkinsons)
  - Post neurosurgical procedures
  - For acoustic neuroma
  - For trigeminal neuralgia
  - Other surgical injury to the trigeminal nerve
- *Ocular*
  - Post-herpes infections (herpes simplex and herpes zoster)
  - Other infections e.g acanthamoeba with nerve damage related to keratoneuritis
  - Chemical and physical burns
  - Abuse of topical anaesthetics
  - Drug toxicity (timolol, betaxolol, diclofenac sodium, sulphacetamide 30%)
  - Chronic ocular surface injury or inflammation

- Ocular surgery
  - Alterations of cornea sensitivity have been observed after cataract surgery even if no frank NK has been reported.
  - Laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) have been have been described as possible causes of NK even if in most cases transient. (Wilson and Ambrosio, 2001)
  - Penetrating keratoplasty (PK) and deep anterior lamellar keratoplasty (DALK) can cause some degree of corneal denervation. up to 12 months after surgery ,[6] but NK is not very frequent in after this kind of surgery. (Lin et al., 2014)
  - Collagen crosslinking for keratoconus.[8]also show a frequently transient reduction of corneal sensitivity (Wasilewski et al., 2013)
  - Development or worsening of NK has been frequently associated with vitrectomy for retinal detachment and photocoagulation to treat diabetic retinopathy (Banerjee et al., 2014)
  - Postsurgical or laser treatment (trauma of ciliary nerves) Routine, single session, indirect laser for proliferative diabetic retinopathy has also been reported as a possible cause of NK. (Tinley and Gray, 2009)
- Contact lens wear
- Orbital neoplasia
- Corneal dystrophies (lattice, granular)

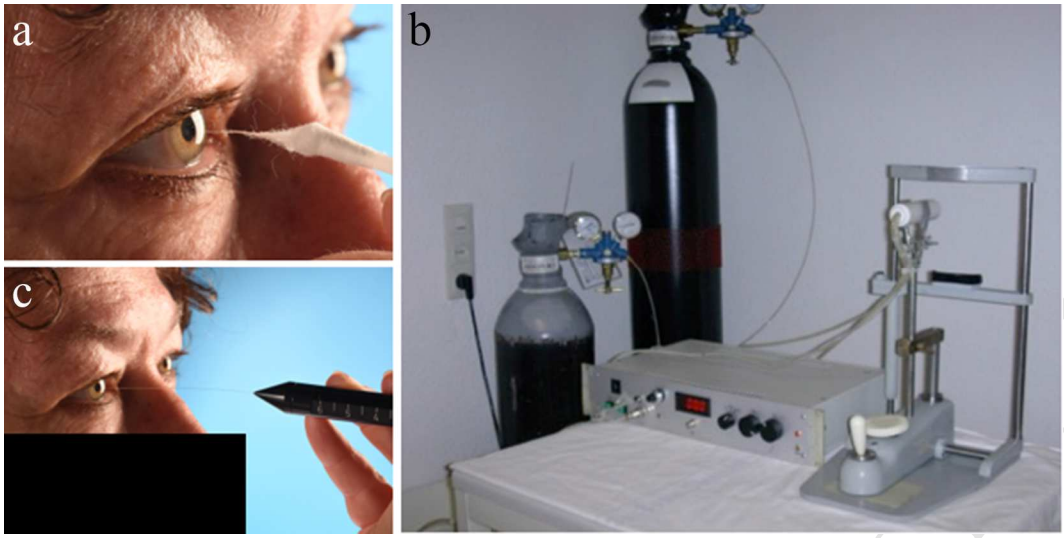
Adapted from author's own publication. (Bonini et al., 2003)

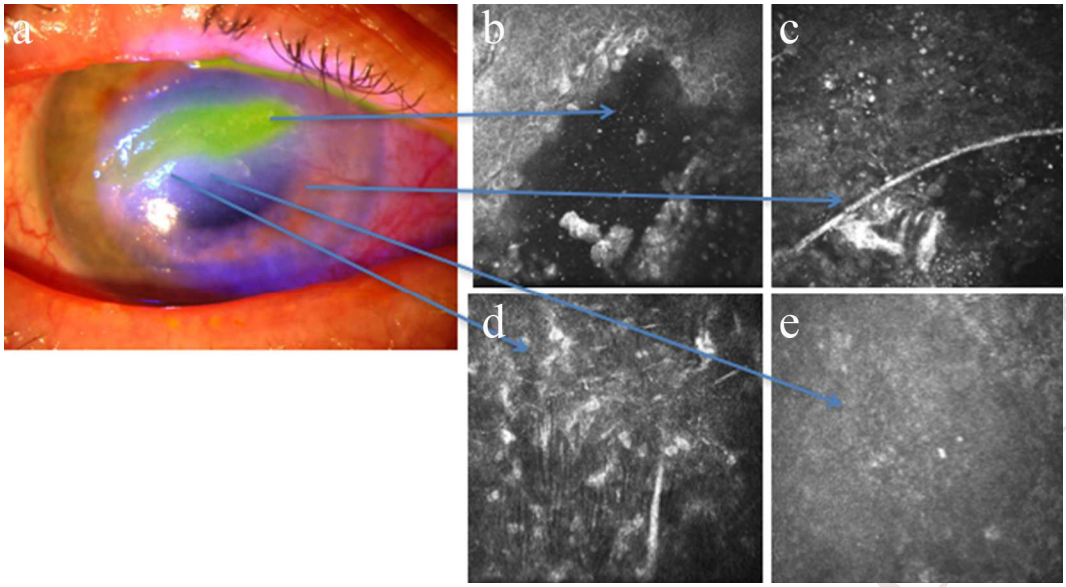
Table 3: Step-ladder approach to management of NK

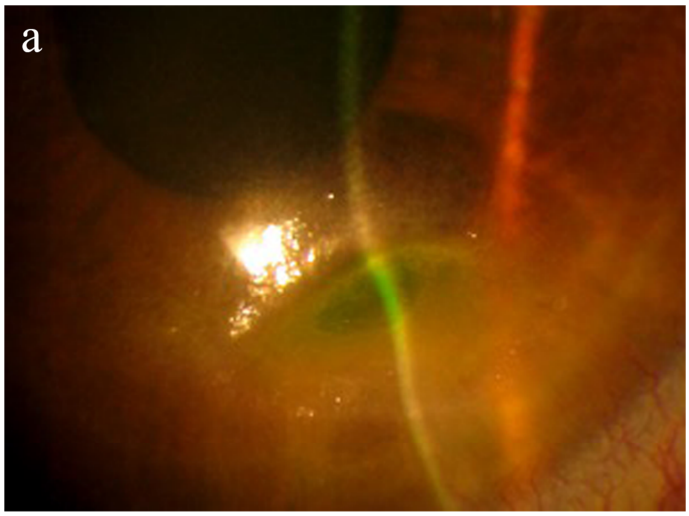
Clinical grade	Therapeutic Options	Intervention Aim
Mild (stage 1)	• Discontinuation of all topical medications especially if containing preservatives. • Evaluation of side effects of systemic therapies such as neuroleptic, antipsychotic, and antihistamine drugs. • Treat concurrent ocular surface problems, especially infection of ocular surface / lacrimal passage. • Anti-inflammatory therapy if inflammation present (non-steroidal anti-inflammatory drugs can be toxic) • Tear substitution / Administration of topical preservative-free lubricants. • Punctal occlusion. • Correction of lid abnormalities. • Debridement of sick epithelium.	• Improve epithelial quality and transparency. • Stabilise epithelium and avoid epithelial breakdown. • Prevent progression to Moderate grade (stage 2, persistent epithelial defect).
Moderate (stage 2)	***As per Stage 1 and:*** • Prophylactic topical preservative-free antibiotics. • Prevention of melting with Citrate / tetracycline / macrolides (if stromal involvement is threatened) • Recombinant Human (rh)NGF (Cenegermin / Oxervate). • Q10 co-enzyme. • Cacicol 20 / RGTA. • Serum eye drops, platelet-rich plasma • Corneal or scleral therapeutic contact lenses. • Non-surgical Eyelid closure. • Debridement of 'rolled' edges of epithelial defect. • Tarsorrhaphy. • Amniotic membrane transplantation usually single layer as patch. • Conjunctival flaps.	• Promote epithelial healing • Prevent the occurrence/recurrence of the epithelial breakdown • Prevent progression to Severe grade (stage 3, stromal lysis)
	As per Stage 2 and:	

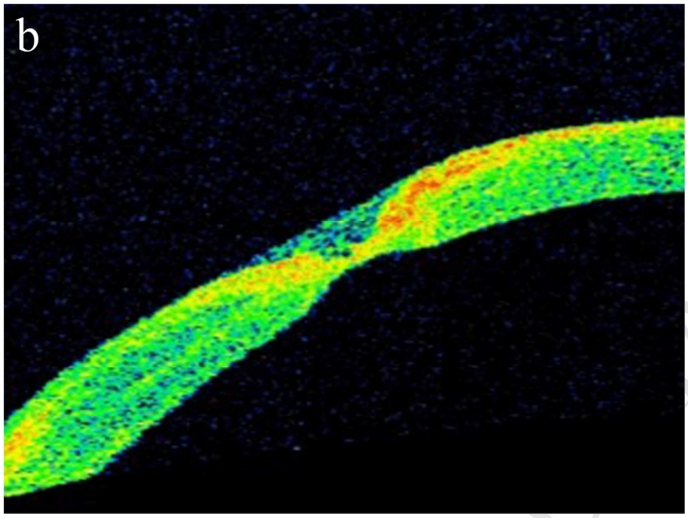
Severe (stage 3)	• rhNGF and RGTA are likely to be of particular help. • Amniotic membrane, multilayer, usually as graft. Can be combined with tarsorrhaphy. • Corneal grafts (tectonic, lamellar or full thickness). ***In the event of perforation*** • Cyanoacrylate tissue adhesive with therapeutic contact lens. • Fibrin glue. • Amniotic membrane graft or corneal grafts.	• Promote corneal healing. • Prevent further corneal stromal lysis and perforation.

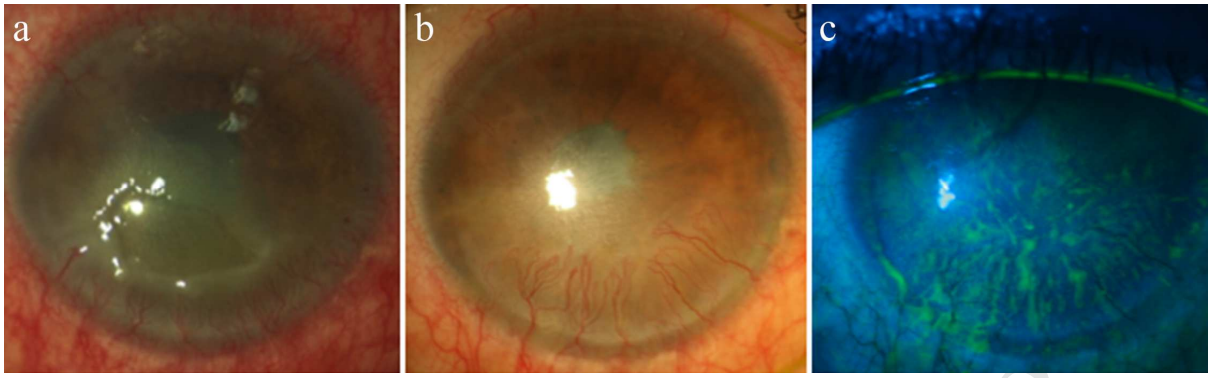
For each grade, all interventions listed may not be required.

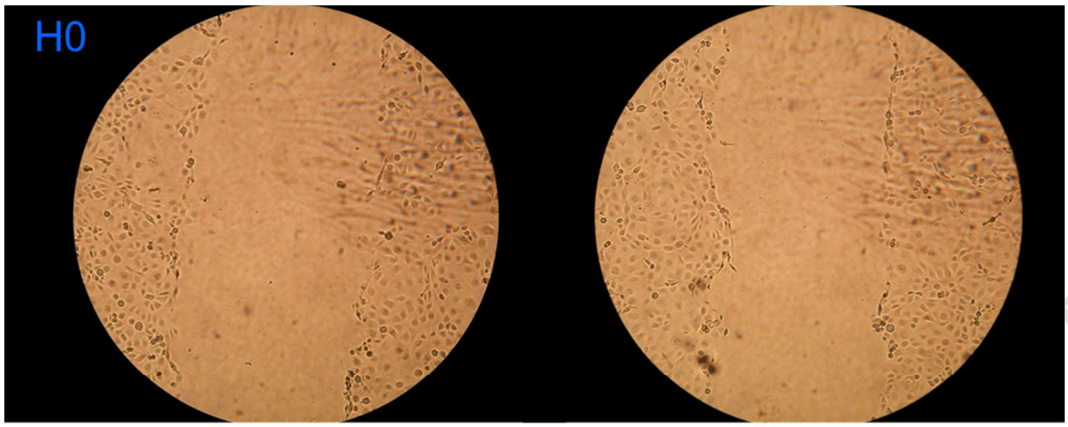


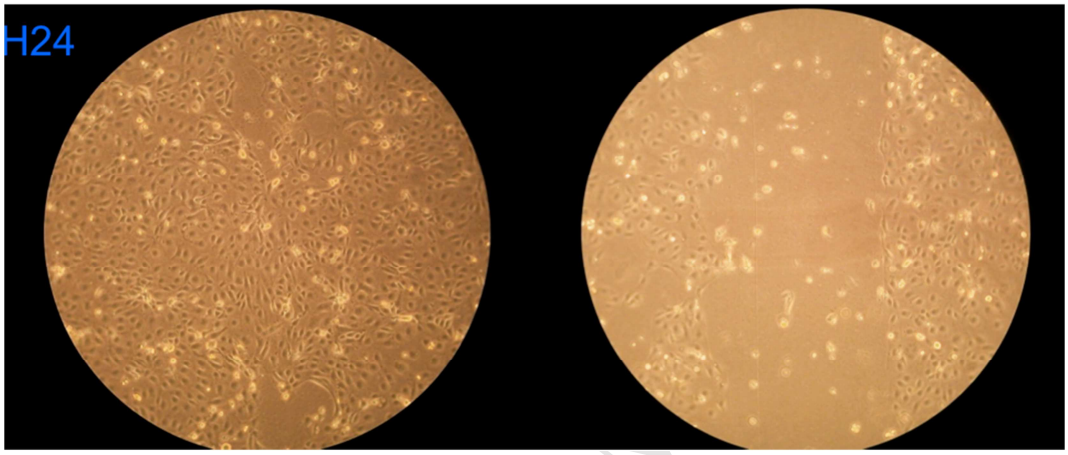


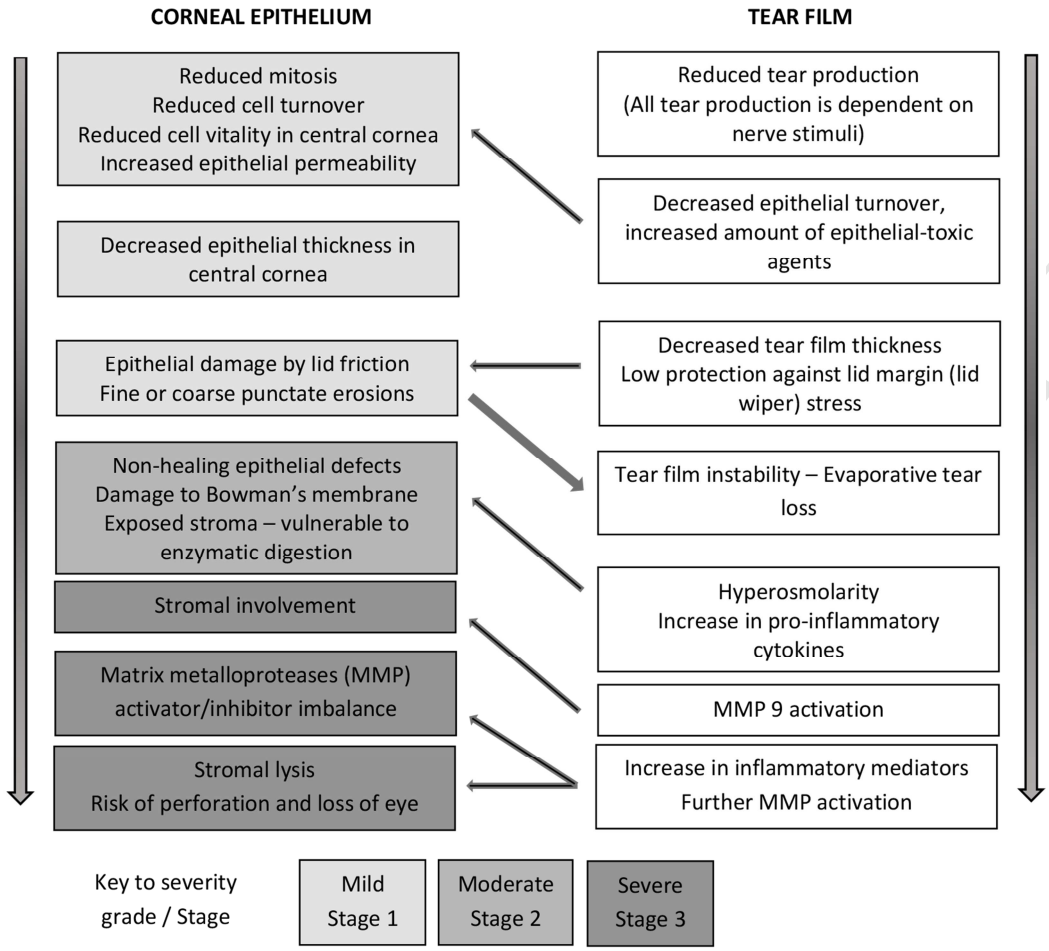


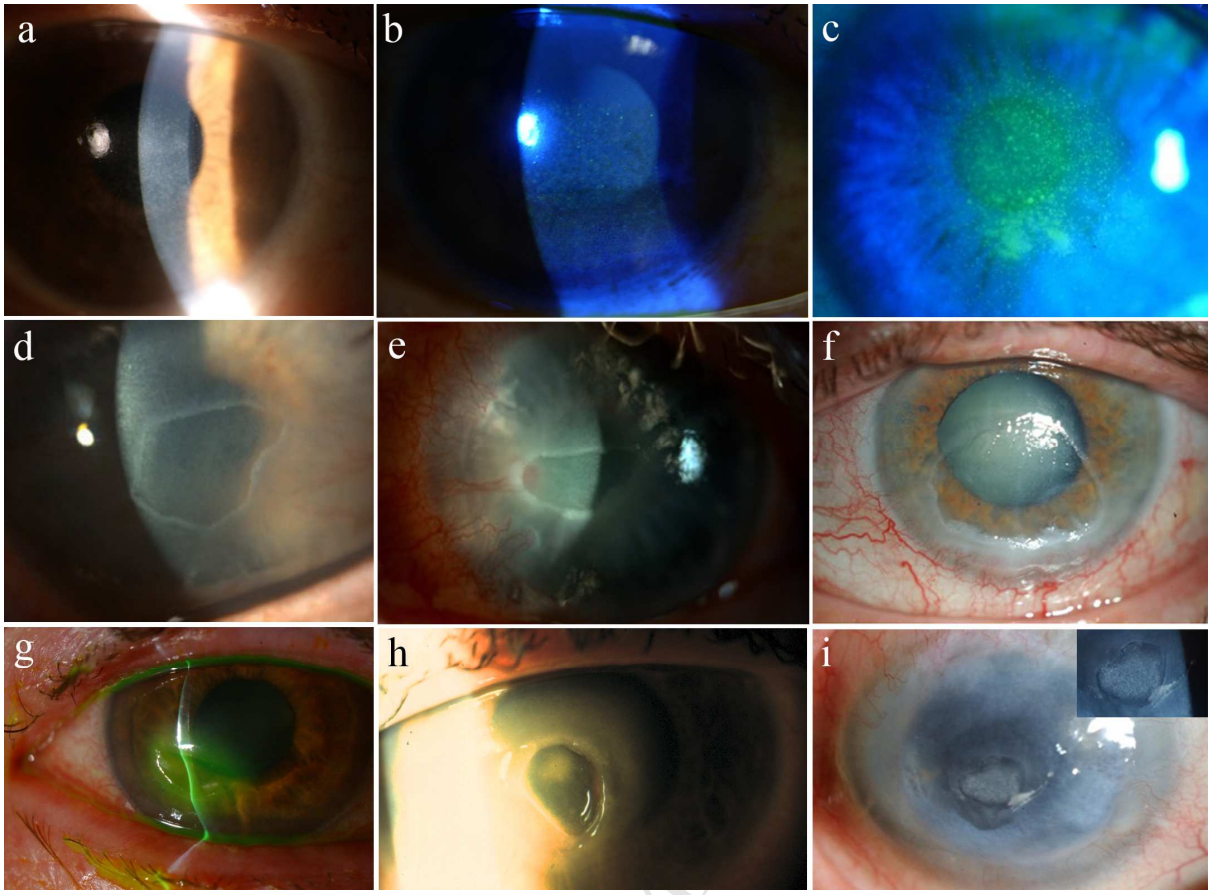


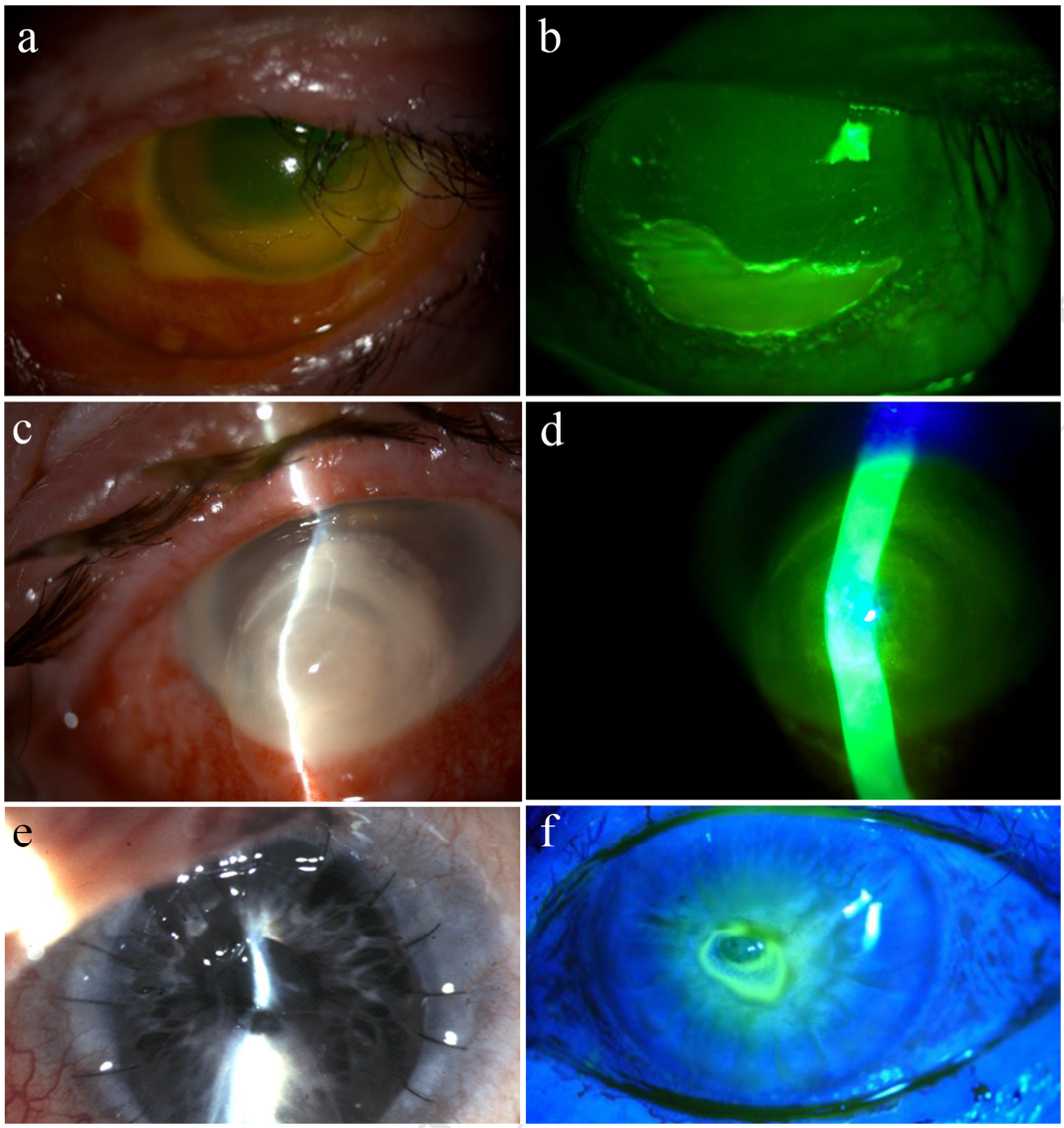


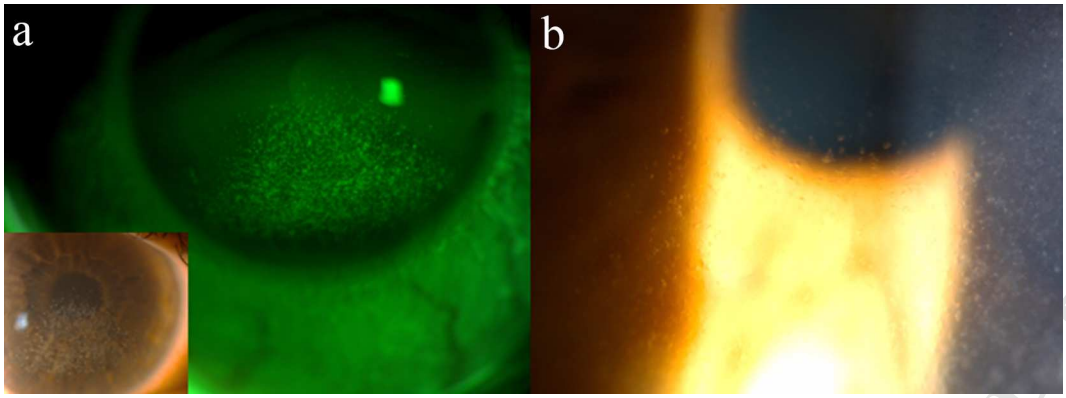


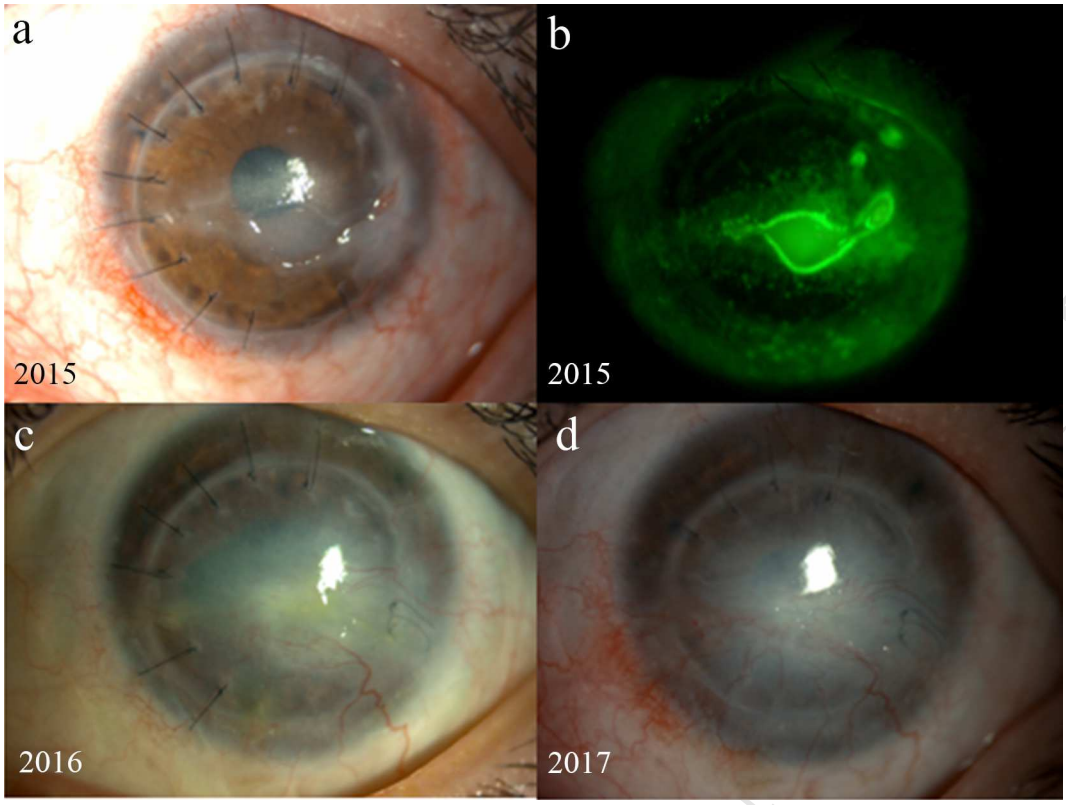


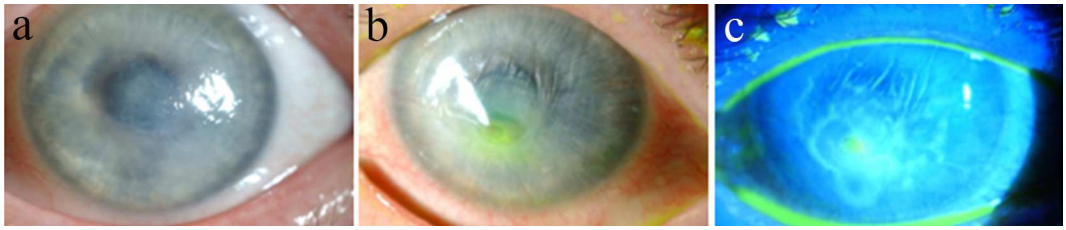


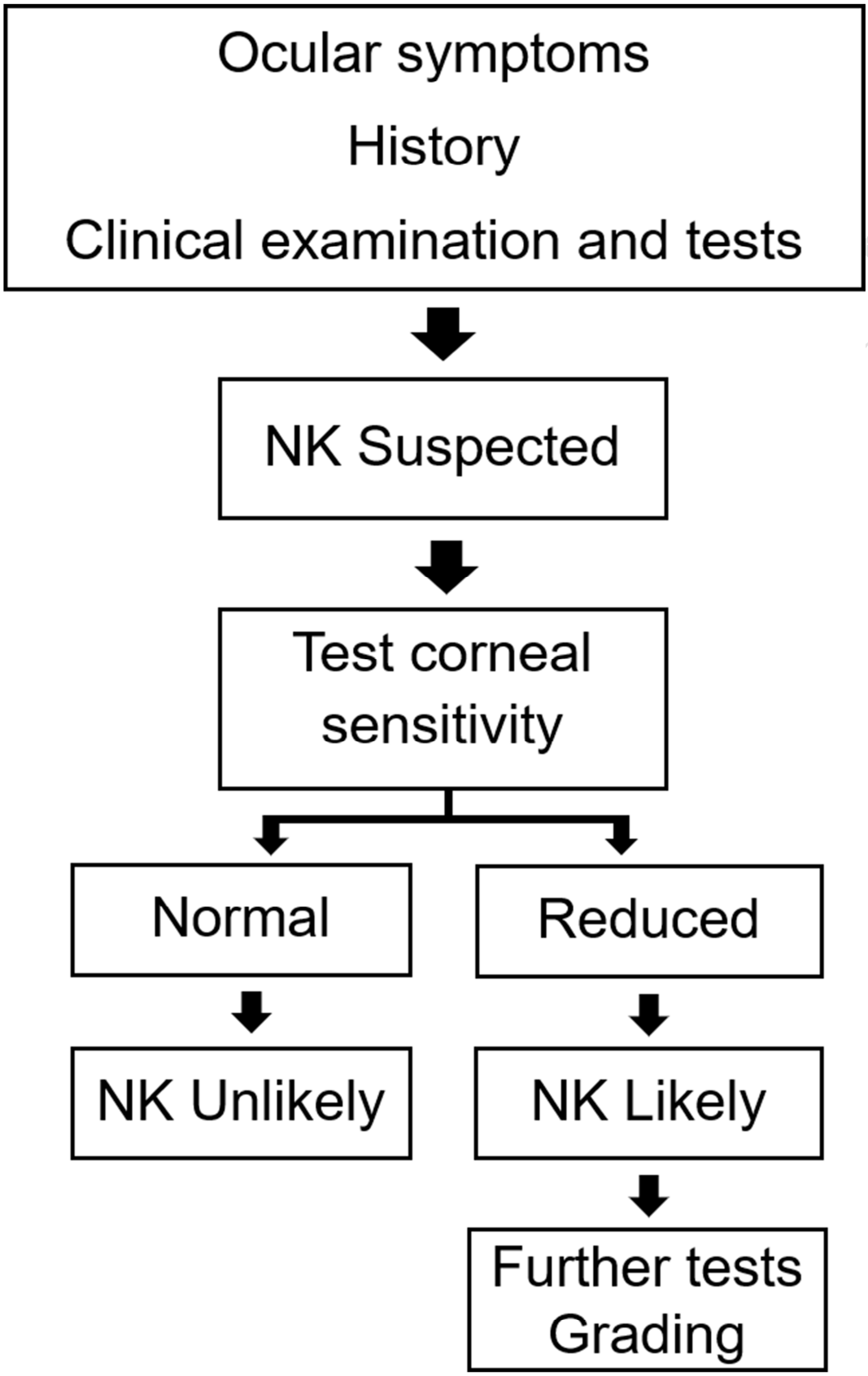


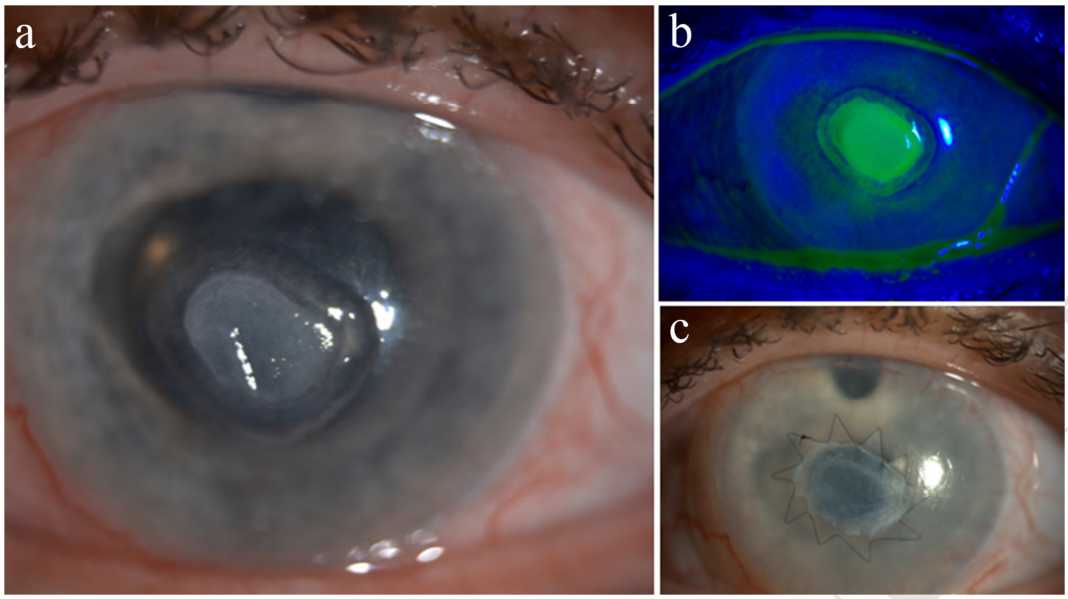


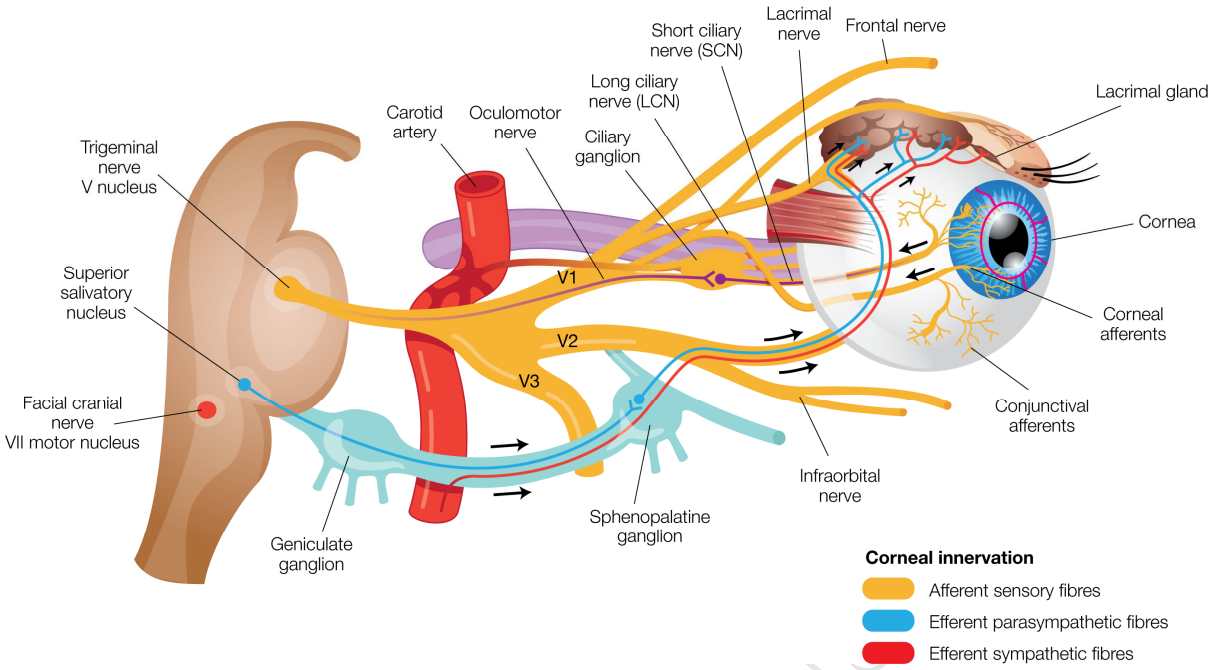


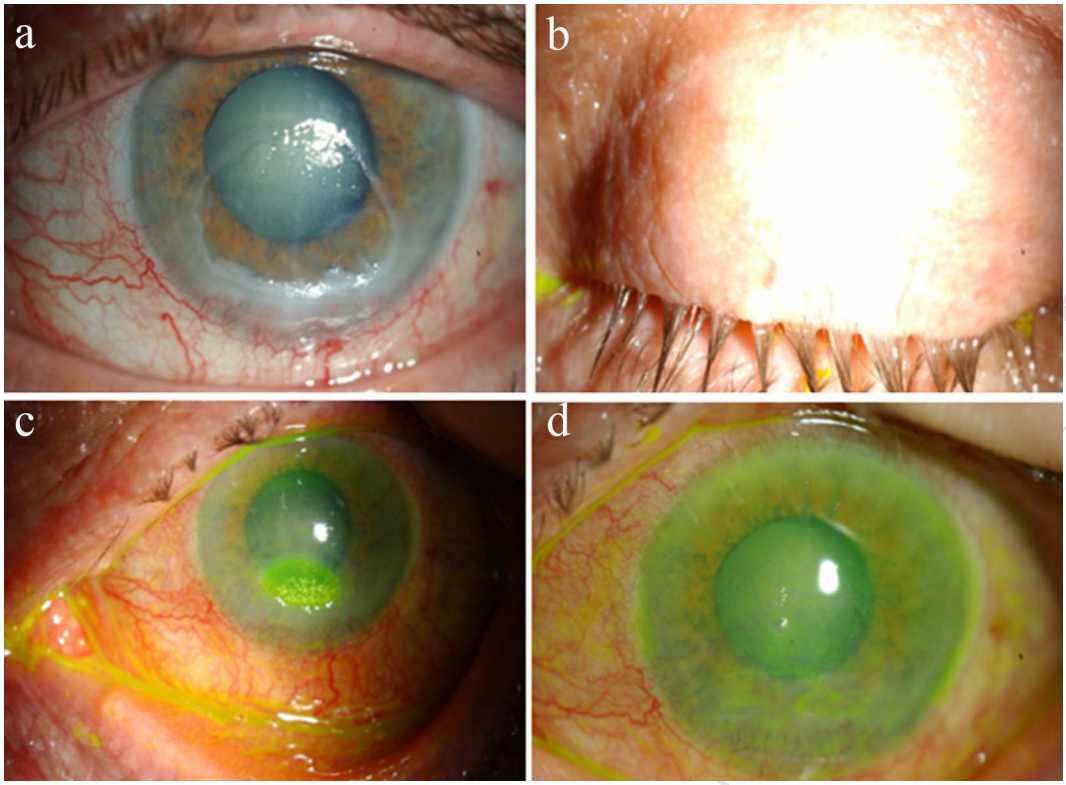


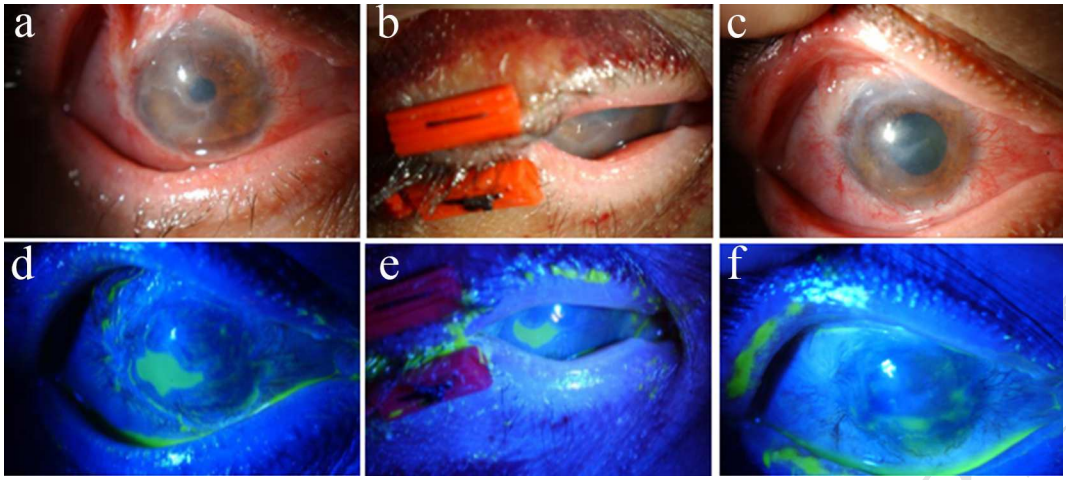


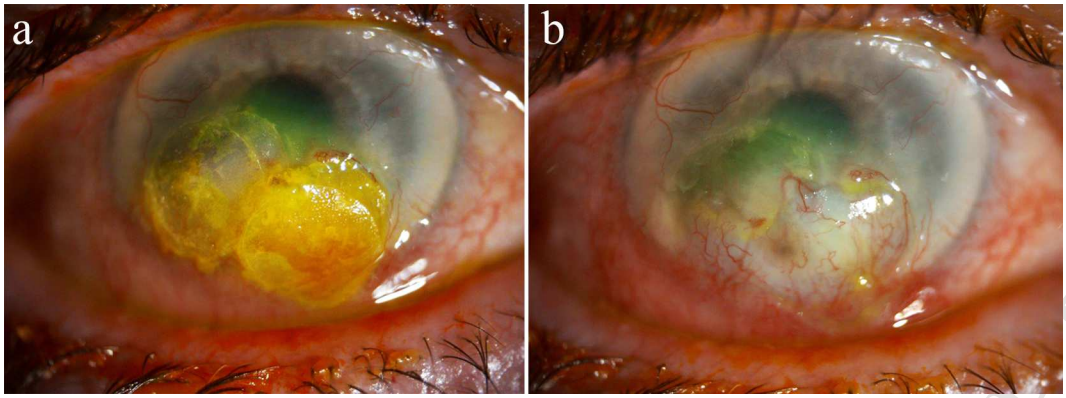

Ocular symptoms
History
Clinical examination and tests
NK Suspected
Test corneal sensitivity
Normal
Reduced
NK Unlikely
NK Likely
Further tests
Grading











Highlights

- Neurotrophic keratopathy (NK) is a relatively rare, ill-understood condition that has a complex etiopathogenesis and can be part of the clinical manifestation of a number of ocular and systemic diseases.
- The dissociation of trophic and sensory nerve functions and the association of hyperalgesia with aberrant nerve re-generation are identified as important issues in understanding NK.
- This study standardizes the nomenclature, definition and classification of NK based on the available evidence.
- The study synthesizes the available evidence on medical, non-surgical and surgical interventions, from the literature and from the experience of experts, into a comprehensive step-ladder approach to the management of NK.