

Neurotrophic keratopathy

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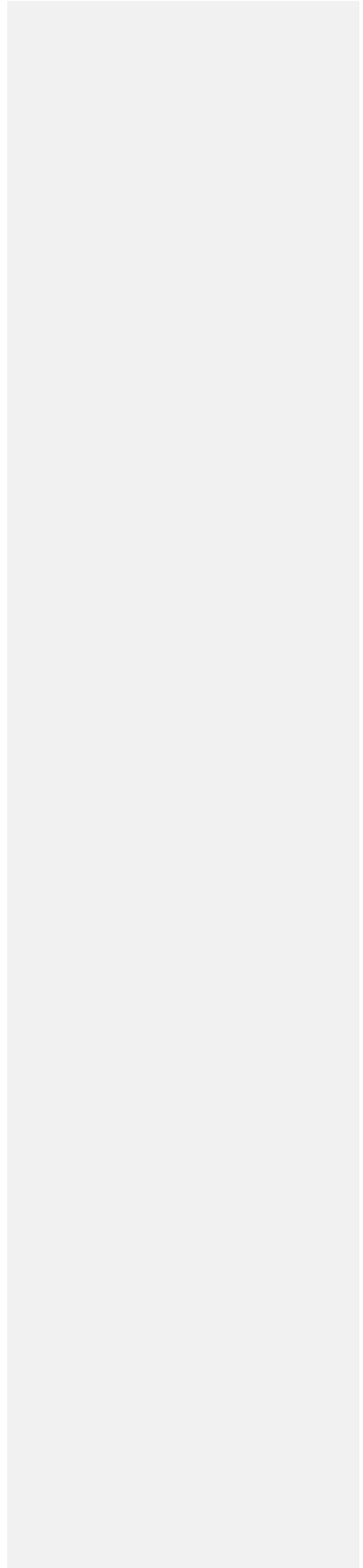
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Title: Neuropathic Keratopathy



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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

1 **Abstract**

2
3 Neurotrophic Keratopathy (NK) refers to a condition where corneal epitheliopathy leading to
4 frank epithelial defect with or without stromal ulceration (melting) is associated with reduced or
5 absent corneal sensations. Sensory nerves serve nociceptor and trophic functions, which can be
6 affected independently or simultaneously. Loss of trophic function and consequent epithelial
7 breakdown exposes the stroma making it susceptible to enzymatic degradation. Nerve pathology
8 can range from attrition to aberrant re-generation with corresponding symptoms from anaesthesia
9 to hyperaesthesia/allodynia. Many systemic and ocular conditions, including surgery and
10 preserved medications can lead to NK. NK can be mild (epithelium and tear film changes),
11 moderate (non-healing epithelial defect) or severe (stromal melting and perforation). Moderate
12 and severe NK can profoundly affect vision and adversely impact on the quality of life. Medical
13 management with lubricating agents from artificial tears to serum/plasma drops, anti-
14 inflammatory agents, antibiotics and anti-proteases all provide non-specific relief, which may be
15 temporary. Contact lenses, punctal plugs, lid closure with botulinum toxin and surgical
16 interventions like tarsorrhaphy, conjunctival flaps and amniotic membrane provide greater
17 success but often at the cost of obscuring sight. Corneal surgery in a dry ocular surface with
18 reduced sensation is at high risk of failure. The recent advent of biologicals such as biopolymers
19 mimicking heparan sulfate; coenzyme Q10 and antisense oligonucleotide that suppress connexin
20 43 expression, all offer promise. Recombinant nerve growth factor (cenegermin), recently
21 approved for human use targets the nerve pathology and has the potential of addressing the
22 underlying deficit and becoming a specific therapy for NK.

23

24 **Key Words:**

- 25 1. Keratitis
- 26 2. Neurotrophic Keratopathy
- 27 3. Trigeminal Neve diseases
- 28 4. Matrix Regenerating agents
- 29 5. Nerve growth factor

30

31 **1. Introduction**

32

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

54 The avascular, squamous corneal epithelium is innervated by a dense mesh of sensory
55 processes derived from the ophthalmic branch of the trigeminal nerve (Muller et al., 2003).
56 These nerve endings are responsible for nociception, cold and pressure sensation. The 7000
57 nerve receptors per square mm of the cornea and their fibres are involved in protecting the
58 cornea from damage by modulating the blink response, stimulating the production of tears and
59 maintaining the cornea in a healthy state through the production of trophic factors (You et al.,
60 2000). Nerve malfunction is the hallmark of neurotrophic keratopathy (NK). Corneal epithelial
61 cells constantly turnover by cell desquamation at the surface, cell regeneration at the limbus and
62 centripetal sliding and migration from the limbus. When the epithelium is injured, cells
63 surrounding the wound migrate onto the wound bed to re-establish cover. This repair requires a
64 controlled and collaborative system of communication between epithelial and neuronal cells to
65 facilitate re-synthesis of the damaged matrix, cell migration and restoration of architecture
66 (Zieske and Gipson, 1986). If epithelial healing is impaired, the exposed stroma becomes
67 vulnerable to enzymatic degradation, melting and eventually perforation; features that represent
68 severe NK (Fini et al., 1998).

69

70 **2. Brief History of Neurotrophic Keratopathy**

71

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

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

94

95 **3. Nomenclature**

96

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

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

114

115 **4. Definition**

116

117 The definition of Neurotrophic keratopathy (NK) has remained consistent over the last
118 couple of decades. At the Cambridge Ophthalmological Symposium in 2003 (Bonini et al.,
119 2003), the following definition was used: "Neurotrophic keratopathy (NK) is a degenerative
120 corneal disease induced by an impairment of trigeminal nerve. Impairment or loss of corneal
121 sensory innervation is responsible for corneal epithelial defects, ulcer, and perforation." The
122 American Academy of Ophthalmology published the following definition in 2008:
123 "Neurotrophic keratopathy (NK) is a degenerative disease of the corneal epithelium resulting

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

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

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

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

157

158 5. Anatomy and Physiology of Corneal Nerves

159 5.1 Anatomy

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

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

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

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

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

195 Autonomic innervation consists essentially of sympathetic nerves from the superior
196 cervical ganglion (SCG). The SCG is located close to the internal carotid artery at the level of the
197 2nd and 3rd cervical vertebrae. It receives preganglionic fibres from neurons located at the level of
198 the 1st and 2nd thoracic spinal nerves. Postganglionic (postsynaptic) fibres from the SCG ascend
199 in the carotid plexus around the internal carotid artery. Fibres destined for the eye leave the
200 carotid plexus in the cavernous sinus and enter the orbit through the superior orbital fissure as
201 the sympathetic root of the ciliary ganglion. Some fibres directly merge with the long ciliary
202 nerves and others pass through the ciliary ganglion, without synapse, to emerge in the short
203 ciliary nerves. Primates including humans have little sympathetic nerve supply to the cornea
204 (Ehinger, 1971; Sugiura and Yamaga, 1968; Toivanen et al., 1987) compared to rabbits and cats
205 where they constitute approximately 15% of the total corneal innervation (Marfurt and Ellis,
206 1993). Rat and cat corneas also receive parasympathetic fibres from the ciliary ganglion (Marfurt
207 et al., 1998; Morgan et al., 1987; Tervo et al., 1979). However, this kind of innervation has not
208 been confirmed in humans. All corneal sensory nerves derive from finely myelinated (A- δ) and
209 unmyelinated (C) axons determined by the size and presence of myelin sheaths in the axon
210 (Felipe et al., 1999). In the human cornea, central stromal axons are unmyelinated and run in the
211 anterior stroma as large bundles parallel to collagen bundles. Most of the axons in these bundles
212 are about 0.5 μ m in diameter. However, few may be as large as 2.5 μ m (Muller et al., 1996;
213 Muller et al., 1997). On the other hand, more than 70% of the axons in rabbit corneas are

214 unmyelinated (Beuerman et al., 1983). The rest are finely myelinated axons that lose their myelin
215 sheath within 1 mm after penetrating the cornea (Lim and Ruskell, 1978; Rozsa and Beuerman,
216 1982; Zander and Weddell, 1951). Myelinated axons are present in the central cornea in some
217 mammals (Rodger, 1950; Whitear, 1960). As soon as they enter the corneal stroma, the nerve
218 bundles lose their perineurium and continue as elongated structures running between the collagen
219 lamellae.

220

221 5.2 *Physiology*

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

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

237 (Acosta, Peral, 2004). The transient receptor potential (TRP) cation channels subfamily V
238 member 1 (TRPV1) plays an important role in sensory transduction in polymodal nociceptors. It
239 is expressed in intraepithelial nerve terminal endings in the corneal epithelium (Alamri et al.,
240 2015; Guo et al., 1999; Murata and Masuko, 2006) and is activated by capsaicin, low pH,
241 noxious heat and hyperosmolarity (Caterina et al., 1997; Davis et al., 2000; Straub, 2014). Acid-
242 sensing ion channels (ASICs) and TRP cation channel subfamily A member 1 (TRPA1) also
243 appear to contribute to chemical sensitivity of corneal polymodal nociceptors (Bandell et al.,
244 2004; Bautista et al., 2013; Callejo et al., 2015).

245 The neuropeptides contained in some polymodal receptors (substance P and calcitonin
246 gen-related peptide – CGRP) maintain corneal homeostasis and integrity by promoting corneal
247 epithelial cell proliferation, migration, adhesion and differentiation (Garcia-Hirschfeld et al.,
248 1994; Reid et al., 1993; Tran et al., 2000). Corneal epithelial cells in turn, release soluble factors
249 (e.g. NGF and GDNF) that promote neurite extension and survival (Chan and Haschke, 1981;
250 Lambiase et al., 2000). Furthermore, the lacrimal gland provides growth factors and nutrients
251 and in turn, its function is influenced by sensory nerves. About 20-30% of peripheral axons
252 innervating the cornea are selective mechano-nociceptors, which respond only to mechanical
253 stimuli at an order of magnitude close to that required for corneal epithelial damage (Belmonte et
254 al., 1991; MacIver and Tanelian, 1993). These mechano-nociceptors are probably responsible for
255 the acute, sharp pain sensation induced by touching or scratching the corneal surface. Piezo2, a
256 newly identified mechanically sensitive ion channel is present in about 30% of corneal sensory
257 neurons in the trigeminal ganglion, but has not been described yet in the intraepithelial nerve
258 terminals (Alamri et al., 2015; Bron et al., 2014). Cold thermoreceptors represent 10-15% of the
259 total population of corneal sensory neurons (Belmonte et al., 2017). They change their activity

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

268 Nerve growth factor (NGF), epidermal growth factor (EGF), glial derived neurotrophic
269 factor (GDNF) as well as brain derived neurotrophic factor (BDNF) are the main agents of an
270 epithelial-nerve cross talk which plays a fundamental role in corneal wellbeing and healing
271 (Muller et al., 2003). NGF, GDNF, their receptors TrkA and GFRA-1, as well as BDNF may also
272 play an important role in maintaining corneal epithelial stem cells in the limbus (Qi et al., 2007).
273 In addition, NGF seems to facilitate innervation of perivascular nerves to regulate blood flow in
274 corneal neovascularization (Matsuyama et al., 2017). Recent evidence indicates, that significant
275 and complex interactions exist between the nervous and immune system. Primary sensory
276 neurons seem to be involved in maintaining the cornea's immune privilege (Belmonte et al.,
277 2017). Moreover, peptidergic polymodal nociceptor terminals with their sensory neuropeptides
278 substance P and CGRP contribute to the inflammatory response following tissue injury
279 (neurogenic inflammation) (Belmonte et al., 2004a): CGRP has immunosuppressive effects,
280 while substance P acts as a potent pro-inflammatory neuropeptide (Micera et al., 2006; Reynier-
281 Rebuffel et al., 1994). Fractalkine (FKN, CX3XL1), produced by primary sensory neurons, plays
282 an important role in the maintenance of corneal well-being, and disturbances in FKN/CX3CR1-

283 signalling may also result in corneal inflammation (Clark, 2014). NGF is a constitutive molecule
284 present and produced in normal human corneas and important for the development and
285 maintenance of peripheral sensory neurons. NGF and/or NGF-receptors TrkA and p75NTR are
286 expressed in many corneal tissues including epithelium, endothelium, keratocytes and nerves, as
287 well as by bone marrow (BM) derived cells present in the cornea (Lambiase et al., 2000; Sarkar
288 et al., 2013). Tear NGF is increased after both photorefractive keratectomy and laser in situ
289 keratomileusis (Lee et al., 2005). In addition, some semaphorins such as Sema7A and VEGF-A
290 act as neurotrophic factors in the cornea and are able to influence inflammatory events (Li et al.,
291 2011; Namavari et al., 2012; Takamatsu and Kumanogoh, 2012). T-cell-dependent inflammation
292 involving IL-17, neutrophils, platelets, and VEGF-A seems to promote corneal nerve
293 regeneration (Li et al., 2011; Namavari et al., 2012; Takamatsu and Kumanogoh, 2012).

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

298

299 **6. Incidence and prevalence**

300

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

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

307 There is a paucity of information in the literature regarding the prevalence and incidence of
308 NK. The best evidence available (Sacchetti and Lambiase, 2014) is based on extrapolation from
309 the two most common conditions associated with NK, which are herpetic keratitis (incidence of
310 1.22/10,000) and post-surgical nerve damage (incidence of 0.02/10,000); as being below
311 1.6/10,000. More specifically, NK develops in an average of 6% of herpetic keratitis cases,
312 which has a prevalence of 149/100,000 (Labetoulle et al., 2005) and in 12.8% of herpes zoster
313 keratitis cases, which has a prevalence of 26/100,000 (Dworkin et al., 2007). Other than herpetic
314 keratitis, the most common cause of NK is neurosurgical intervention to treat trigeminal
315 neuralgia that damages the trigeminal nerve (post-surgical incidence of 2.8%), which has a
316 prevalence of 1.5/10,000, and an estimated prevalence of NK after neurosurgical procedure of
317 0.02/10,000 (Bhatti and Patel, 2005). Unfortunately, no epidemiological data can be found in the
318 literature for a number of other common conditions known to cause NK such as chemical burns,
319 diabetes, contact lenses and less frequent causes such as space occupying intracranial masses,
320 multiple sclerosis and leprosy.

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

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

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

334

335 **7. Aetiology and Pathogenesis of Neurotrophic Keratopathy**

336 *7.1 Etiology of Neurotrophic Keratopathy*

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

349 0-30, SD 9.73) in the LSCD/NK eyes and 59.13 (range 50-60, SD 2.881) in the fellow normal
350 eyes. The difference between the LSCD/NK and fellow eyes was statistically significant
351 ($p < 0.0001$, Wilcoxon rank test). A summary of the common causes of ocular surface nerve
352 damage is given in table 2.

353

354 7.2 Pathogenesis of NK

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

365

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

371 by corneal nerve fibres (De Haas, 1962; Duke-Elder and Leigh, 1965; Heigle and Pflugfelder,
372 1996; Paton, 1926; Wilson and Ambrosio, 2001). In most cases it is probably a combination of
373 these factors, with a neurotrophic deficit playing a major role.

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

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

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

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

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

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

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

439 | micropipette tip under an inverted microscope (Fig. 24). 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 microplacae. 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

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

465 NK related to herpes related keratitis is an enigma. Recurrent HSK results in persistent
466 epithelial defects in the cornea ipsilateral to the affected trigeminal pathway. This is associated
467 with hyperosmolarity, reduced tear break up time, reduced tear secretion (Schirmer I test) and
468 reduced sensations not only in the affected eye but also in the unaffected eye (Rousseau et al.,
469 2015; M'Garrech et al., 2013; Jabbarvand et al., 2015). Unilateral herpes simplex and herpes
470 zoster keratitis is associated with bilateral loss of corneal nerve receptors and dendritic cell
471 infiltration (Hamrah et al., 2010; Hamrah et al., 2013; Cavalcanti et al., 2018). The findings are
472 mirrored in studies on rabbits with unilateral trigeminal axotomy, wherein bilateral loss of
473 corneal nerves and immune cell infiltration was demonstrated (Yamaguchi et al., 2016;
474 Yamaguchi et al., 2013). This suggests that unilateral affection of the (central) trigeminal
475 pathways could trigger bilateral responses probably via neurogenic inflammation. In humans,
476 though clinical manifestations are predominantly unilateral it is possible that subclinical NK is
477 present in the other eye as well, in some cases. Whether amelioration of signs and symptoms by
478 treatment of the affected eye leads to resolution of changes in the other eye remains to be seen.

479

480 7.3 Natural History of NK

481 When cornea sensitivity is impaired or lost, a sequence of events is triggered at the ocular
482 surface leading to NK and its consequences (Fig. 32).

483 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

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

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

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

512

513 **8. Clinical presentation and classification**

514 Patients with NK will present in the early stage of the disease with symptoms of dryness,
515 photophobia, and inability to read for a prolonged period of time due to epithelial changes and
516 instability (see pathogenesis), impaired quality of vision and reduced blink. Symptoms are
517 usually worse in the morning or in the presence of aggravating factors such as air conditioning,
518 air travel, draught of hot air from car heating or prolonged use of some computers (draught of hot
519 air from computer fans and reduced blinking associated with mental concentration) (Dua et al.,
520 2014). Paradoxically, with worsening or severe disease, the symptoms of pain and discomfort
521 may be less or absent due to sensory dullness related to hypoaesthesia or anaesthesia of the
522 cornea. Symptoms of visual impairment appear when the central cornea is significantly
523 involved. Clinical signs relate to those associated with the underlying condition and those due
524 NK, usually a combination of the two.

525

526 *8.1 Signs related to NK*

527

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

530 keratitis SPK). The blink rate can be reduced and irregular (normal 17 blinks per minute) (Bentivoglio et
531 al., 1997). The cornea reflex (lustre) becomes dull and the epithelium appears cloudy with epithelial
532 irregularities. In NK related to laser refractive surgery (Ocular surface syndrome) (Alio et al., 2007;
533 Ambrosio et al., 2008), SPK is centrally located and can wax and wane (Fig. 43 a-c). With increasing
534 severity, the epithelial erosions coalesce and become larger (coarse erosions) and a frank epithelial defect,
535 usually centrally located, appears (Fig. 43 d-f). Attempts at healing of the defect are slow and often
536 incomplete. The area of the defect changes as the epithelium heals and breaks down repeatedly.
537 Eventually the defect becomes permanent (persistent epithelial defect, PED) with smooth or rolled and
538 opaque edges. The epithelium around the perimeter of the defect is loosely attached to the underlying
539 Bowman's layer as evidenced by the sub-epithelial seepage of fluorescein dye, beyond the edge of the
540 defect. The exposed stroma is vulnerable to the effect of proteases resulting in melting, that can lead to
541 perforation (Fig. 43 g-i). The ulcer is usually sterile however secondary microbial infection can occur
542 which can lead to rapid progression of melting and perforation. (Fig. 54). Stromal involvement can also
543 manifest as edema, striae and Descemet's folds. Cells in the anterior chamber can be seen and frank
544 hypopyon should be carefully evaluated and followed since it can be sterile or a sign of secondary
545 infection. In vivo confocal microscopy examination, in the presence of frank corneal hypoesthesia, can
546 show an intact sub-basal plexus (pre-ganglionic affection of the Vth nerve) with a relatively better
547 prognosis (Dhillon et al., 2016). In the majority of cases however, the sub-basal plexus is deficient.

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

553

554 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

582 Stage 3 is characterised by:

- 583 • Stromal lysis/melting
- 584 • May result in perforation

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

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

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

613 Epithelial disturbance, frank non-healing epithelial defect and stromal lysis usually but
614 not necessarily follow sequentially (Figs. 65-87) . All other clinical signs of NK are variable and
615 do not appear or progress sequentially as is often determined by the underlying condition for
616 example in a case of chemical burn the patient may present with severe NK without having
617 progressed through NK of mild and moderate severity. It is important to understand the
618 difference between an abrasion and an ulcer as both are technically 'epithelial defects'. Abrasion
619 implies the rubbing or scraping away of cells from the surface of an area of the cornea, skin or
620 mucous membrane; whereas an ulcer is a breach of the continuity of the epithelium of any of the
621 above mentioned tissues, due to sloughing related to inflammation and tissue necrosis. Abrasions
622 generally heal rapidly while ulcers take longer to heal, festering as 'non-healing epithelial
623 defects'.

624

625 **9. Diagnosis and Differential diagnosis**

626

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

631 *9.1 Ocular symptoms*

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

639

640 *9.2 Clinical History*

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

647 9.3 Examination

648 9.3.1 Neurological Examination

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

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

660 9.3.2 Ophthalmic Examination

661 9.3.2.1 External exam (eyelids and conjunctiva) *Eyelids*

662 Eyelid function is critical to the prognosis of neurotrophic keratitis and progression of
663 advanced disease. Lid features to note are ectropion, entropion, misdirected lashes or ptosis
664 (oculomotor nerve damage or mild ptosis in the presence of corneal infection). Lid scarring may
665 be present secondary to removal of periocular infiltrative tumors or chemical or thermal burns.
666 The conjunctivae in NK patients generally show a lack of conjunctival injection i.e. 'white' eye.
667 The presence of 'red eye', however, would indicate the presence of co-existing inflammation

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668 usually related to secondary infection. Subconjunctival fibrosis may be present, which could be
669 associated with chronic autoimmune disease and/or severe dry eye.

670 9.3.2.2 *Slit lamp examination*

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

680 9.4 *Vital Staining*

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

689 The above account illustrates that, as with any ocular assessment, with NK too, it has to be
690 thorough and complete and not restricted to the cornea or ocular surface.

691 9.5 *Diagnostic tests*

692 9.5.1 *Corneal Sensation*

693 Assessment of corneal sensation is fundamental to the diagnosis of NK. The algorithm
694 for diagnosis is given in the diagram (Fig. 98). Clinically, corneal sensation is assessed by using
695 a 'wisp' of cotton applied to both corneas (Fig. 109a). Patient's reaction is noted and compared
696 between the eyes. NK patients typically show reduced blinking/sensation to the stimulus.
697 Corneal sensation is reduced or normal, if it is normal then NK unlikely. Some authors have
698 stated that an absence of the nasal-lacrimal tearing reflex along with ipsilateral loss of sensation
699 in the nasal mucosa presents a high risk for subsequent neurotrophic corneal ulceration, so there
700 may be a basis for testing this.

701 Corneal sensation can be (semi)quantitatively measured by the Cochet-Bonnet
702 aesthesiometer or the Belmonte non-contact gas aesthesiometer (BNGA) (Fig. 109b and 109c).
703 The former is a contact instrument and the latter is not. With the Cochet-Bonnet aesthesiometer,
704 corneal sensitivity is assessed observing the patient's subjective reaction to different lengths of a
705 protruding nylon filament applied to the cornea which is extended from 60mm to 5mm with a
706 corresponding change in force from 11 to 200 grams/mm. After touching the cornea in the
707 quadrant to be tested, pressure is applied on the filament to induce a gentle bend. At this point
708 the patient should appreciate the touch of the filament tip. The length is reduced in 5mm steps
709 until the patient appreciates the touch. The longer the length at which the patient feels the touch

710 of the filament, the higher the corneal sensitivity. Measurements are performed in each quadrant
711 of the cornea and data recorded accordingly (Golebiowski et al., 2011).

712 The BNGA works by stimulating the cornea with a calibrated gas emission from an
713 injector kept close to the cornea and subsequent blink response. The BNGA is mounted on a slit-
714 lamp (similar to an applanation tonometer) with the gas injection tip kept perpendicular to the
715 cornea, the subject is instructed to look at a fixation target at 3 m, and the injection tip is kept
716 5mm away from the surface, (the distance is measured with a transparent ruler). Subjects are
717 instructed to close and open their eyes just before triggering the stimulus. By varying the flow,
718 temperature and composition of gas (CO₂ concentration) this device can assess different
719 components of corneal sensation i.e. mechanical, chemical and thermal sensitivity. The
720 technique has been found to be safe and reproducible and its ‘non-contact’ nature makes it safer
721 than contact methods (Belmonte et al., 1999; Teson et al., 2012).

722 9.5.2 *Imaging Corneal Nerves*

723 In vivo confocal microscopy (IVCM) (Fig. 110) allows qualitative and quantitative
724 assessment of corneal nerves in health and disease. Nerve density, tortuosity, angulation,
725 thickness and reflectivity are assessed using image analysis programmes. IVCM has been used to
726 detect corneal nerves changes in a variety of conditions such as keratconus, bullous keratopathy,
727 diabetic neuropathy and herpes simplex keratitis (Al-Aqaba et al., 2011a; Al-Aqaba et al., 2011b;
728 Cottrell et al., 2014; Messmer et al., 2010). In diabetic neuropathy, analysis of corneal nerve
729 density and morphology has demonstrated a correlation between reduction in fibre
730 density/branching and severity of somatic neuropathy. The method is sensitive enough to detect
731 significant structural abnormality in the corneal nerves of patients deemed to have mild diabetic
732 neuropathy by conventional tests and can help detect onset of diabetic peripheral neuropathy

733 prior to clinical manifestation. In pre-ganglionic (trigeminal ganglion) lesions and partial
734 ganglion lesions, corneal sensitivity can be absent or diminished but IVCM demonstrates a
735 normal sub-basal plexus with mild NK. In post-ganglionic or complete ganglionic lesions the
736 sub-basal plexus is attenuated or lost, corneal sensations are reduced and the risk of developing
737 moderate to severe NK is high (Dhillon et al., 2016). Accurate imaging and quantitative analysis
738 of images of sub-basal and stromal nerves can be affected in corneas presenting with severe NK
739 due to the possible influence of concurrent stromal oedema, infiltration, melting or scarring.

740 9.5.3 Anterior Segment Optical Coherence Tomography (ASOCT)

741 Fourier domain ASOCT can demonstrate corneal nerve abnormalities (radial
742 keratoneuritis) such as during active acanthamoeba keratitis (Yamazaki et al., 2014) and can be
743 used to assess treatment response. However, current instrumentation has insufficient resolution
744 to resolve corneal nerve architecture changes where the sole abnormality is loss of corneal
745 sensation. ASOCT comes in handy to assess corneal thickness changes as may occur during
746 moderate to severe NK; providing both morphometric and qualitative data. ASOCT can be used
747 for measuring the depth of stromal ulcerations and stromal thickness changes occurring over
748 time in NK (Fig. 124), facilitating the diagnosis of cases at risk of perforation and improving the
749 follow-up analysis after surgical tectonic grafts of amnion or corneal transplantation (Nubile et
750 al., 2011).

751 9.6 Differential diagnosis

752 Several chronic diseases can lead to NK and others can mimic NK, the key distinction
753 being the alteration/absence of corneal sensation. The terms primary and secondary NK have
754 been used as descriptors but there ~~is~~are no differences clinically in the corneal manifestations

755 once the process is driven by loss of trophic and sensory function. Systemic causes such as
756 trigeminal nerve damage from tumour, trauma or surgery, or neuropathy associated with diabetes
757 and multiple sclerosis can be considered as examples of primary NK whereas damage to the
758 corneal innervation from direct insult to the cornea such as following viral infections, corneal
759 transplantation or refractive surgery, can be considered as examples of secondary NK. Central
760 and peripheral NK are term that also reflect the site of lesions that can cause NK, analogous to
761 primary and secondary NK. In central NK corneal and conjunctival sensations are likely to be
762 affected compared to peripheral NK where only corneal sensations are likely to be
763 predominantly affected. In the majority of cases NK is a unilateral disease. Where the cause is
764 diabetic neuropathy or multiple sclerosis it can be bilateral.

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

769

770 **10. Management**

771 Left untreated, NK can evolve into a devastating condition culminating in anatomical loss
772 of the eye. Short of this, loss of vision is common even with treatment. Management of NK can
773 be divided into medical management, non-surgical intervention and surgical management. These
774 can be considered in a step-ladder approach according to NK stage/severity but are not exclusive
775 as often a combination of options may need to be considered. The objective of treatment is to
776 arrest progression and reverse NK changes that have occurred at the time of presentation. In mild

777 NK (stage 1), the objective is to prevent epithelial breakdown and encourage healing of epithelial
778 erosions. In moderate NK (stage 2), the objective is to encourage re-epithelialisation of the
779 denuded stroma and prevent progression to stromal melting. In severe NK (stage 3), the objective
780 is to prevent perforation and promote healing. Throughout the course of NK maintenance of
781 comfort and optimizing vision are also therapeutic considerations.

782 *10.1 Medical Management of NK*

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

790 *10.1.1 Treatment of concurrent inflammation*

791 *10.1.1.1 Infection*

792 Culture or PCR of forniceal swabs to identify common and unusual pathogens that may
793 be present in abundance on a compromised ocular surface driving inflammation through
794 activation of innate immune responses, is essential (Kugadas and Gadgeva, 2016). Identified
795 organisms (bacteria, fungi, and viruses) should be treated. However, when infection is suspected
796 but cultures are negative, empirical treatment with Azithromycin 1g orally for 3 days is
797 advised. ~~Due to false negatives, empirical treatment with Azithromycin 1g orally for 3 days is~~

798 ~~advised.~~ For persistent epithelial defects, secondary infection delaying healing should be
799 excluded and the eye treated with broad spectrum topical antibiotics. Wherever possible, toxic
800 aminoglycosides such as gentamicin should be avoided unless sensitivities dictate otherwise.
801 Topically administered quinolones can also be toxic to the ocular surface (Ayaki et al., 2012;
802 Mencucci et al., 2011; Walter and Tyler, 2001). Drug toxicity should be suspected when initial
803 clinical improvement changes to clinical worsening and increased inflammation. The ‘up-down’
804 test, where in the upper bulbar conjunctiva appear white compared to the lower injected bulbar
805 and fornicial conjunctiva, is a useful early indicator of drug toxicity. (Dua et al., 2012).

806 10.1.1.2 *Minimise ocular irritants and conservative treatment*

807 Awareness of iatrogenic causes of ocular toxicity is critical (Dart, 2003). All preserved
808 therapy should, wherever possible, be discontinued. Detection of corneal deposits such as
809 fluoroquinolone crystals (ciprofloxacin, ofloxacin) (Claerhout et al., 2003; Mitra et al., 2007), or
810 hydroxyapatite formation due to a combined effect of hyaluronates and phosphates (Bernauer et
811 al., 2006a), or calcific deposits after the use of steroid-phosphate (Schlotzer-Schrehardt et al.,
812 1999), retinoic acid (Avisar et al., 1988), or lubricant phosphates (Bernauer et al., 2006b) should
813 lead to clinical approaches that minimise potentially damaging drug/chemical precipitation
814 reactions on the ocular surface.

815 Patients should also be advised to refrain from using irritating peri-ocular cosmetics,
816 increase humidity by wearing protective moisture chamber wrap-around glasses or goggles, and
817 consider increasing dietary omega-3 fatty acids (Eicosapentaenoic acid (EPA)) or linoleic acid
818 and gamma-linolenic acid (Flaxseed oil). Fish oils have been shown to provide modest benefit to

819 the ocular surface by inhibiting pro-inflammatory mediators (Prostaglandin E2, Leukotriene B4,
820 IL-1 and TNF α) (Kangari et al., 2013).

821 *10.1.1.3 Reduce Inflammation*

822 The use of non-preserved topical medications is essential to minimise ocular surface
823 inflammation. Meibomian gland dysfunction should be treated with warm compresses possibly
824 with the aid of proprietary eye-lid warming devices, lid massage and hygiene with diluted
825 sodium bicarbonate or commercially available lid hygiene wipes. The use of matrix
826 metalloproteinases inhibitors in the form of low dose tetracyclines or macrolides is advised. The
827 mainstay is with the use of topical non-preserved glucocorticoids such as prednisolone or
828 dexamethasone (Geerling et al., 2011). Delayed wound healing related to steroid medication,
829 pharmacokinetically only occurs at higher doses or with longer duration of treatment.
830 Nevertheless, inhibition of stromal healing, may increase the risk of corneal stromal melting and
831 perforation, thus their use should be considered with caution. Vigilant screening for steroid-
832 related raised intraocular pressure and optic neuropathy is required. Soft steroids such as
833 medroxyprogesterone 1%-2% and androgens (if available) may ameliorate this risk. Topical non-
834 steroid anti-inflammatory drugs (NSAID) treatment does not improve the healing process.

835 NSAIDs are generally avoided due to their epithelial toxicity and the risk of corneal ulceration
836 ([Guidera et al., 2001](#); [Gaynes and Fiscella, 2002](#); [Lee and Himmel, 2006](#); [Feiz et al., 2009](#)):-

837 Topical ciclosporin is only licensed for use in primary or secondary dry eye disease.
838 Ciclosporin 0.1% (Ikervis® SDU) is licensed for severe keratitis of dry eye disease not
839 responding to ocular lubricants. In the US and Far East, Ciclosporin 0.05% (Restasis®) may be a
840 useful alternative. PADciclo 0.06% is currently undergoing clinical trials. The veterinary
841 preparation, Ciclosporin 0.2%, (unlicensed, Optimune®) may also be considered if an ointment

842 with lubricating properties is required. Although tacrolimus 0.03% to 0.1% has been shown to be
843 effective in combating ocular surface inflammation, its use in the context of NK has not been
844 studied (Fukushima et al., 2014; Kiiski et al., 2014).

845 *10.1.2 Tear substitution*

846 Ocular lubricants reduce biomechanical shear forces and dilute pro-inflammatory
847 mediators in the tear film thereby promoting epithelialisation. They are generally classified
848 according to viscosity ranging from low to high and are summarised in table 4. Due to the
849 toxicity associated with preservatives (Baudouin et al., 2010; Geerling et al., 2001; Gomes et al.,
850 2017), unpreserved lubricants are prescribed, many of which can be bought over the counter by
851 the patient. Carmellose agents are cytoprotective (Garrett et al., 2007), and hyaluronate ligation
852 to CD44 expressed on injured ocular surface epithelial cells, deliver anti-inflammatory properties
853 in addition to facilitating epithelial wound healing (Gomes et al., 2004). Other compounds such
854 as guar gums, liposomes and soybean/mineral oil combinations, help stabilise the phospholipid
855 layer whilst other agents confer osmoprotection (glycerine, L-Carnitine, erythritol, threalose). In
856 patients with filamentary keratitis, mucolytics (acetylcysteine 5-10%) may be beneficial. Recent
857 clinical trials indicate promising results for the use of diquafosol 3% (a P2Y2 receptor activator
858 that improves mucociliary clearance and mucin production) and rebamipide 2% (that stimulates
859 transmembrane mucin MUC16 biosynthesis) in multifactorial dry eye disease, although the exact
860 benefit for NK is yet to be determined. Alternative non-preserved lubricants such as saline 0.9%
861 (that has no excipients) or balance salt solution should be considered in resistant cases.

862 Nutritional tear substitutes (serum eye drops) are considered when all treatment options have
863 been exhausted. Unlike other pharmaceutical tear supplements, nutritional substitutes contain

864 substances that are also present in natural lacrimal tears and support ocular surface epithelial
865 growth and regeneration. These include growth factors (epidermal growth factor and
866 transforming growth factor beta), Vitamins (A, C), glucose, natural antimicrobials (surface IgA,
867 defensins, lysozyme), and proteins involved in wound healing (fibronectin) (Rauz and Saw,
868 2010). Most commonly used are autologous serum eye drops (Azari and Rapuano, 2015;
869 Semeraro et al., 2014; Turkoglu et al., 2014), but there is a risk of instilling circulating antibodies
870 or pro-inflammatory mediators in patients with systemic diseases e.g. Multiple sclerosis, mucous
871 membrane pemphigoid that have the potential to exacerbate disease processes in the eye.
872 Allogeneic serum eye drops (available in the UK, Germany, New Zealand) are obtained from
873 young healthy male donors and avoid cyclical oestrogen hormone variances that may have pro-
874 inflammatory effects on the surface of the eye. Human umbilical cord serum has been shown to
875 contain higher levels of growth factors, including nerve growth factor, and other constituents
876 similar to those in tears, and has been used to treat a variety of ocular surface conditions
877 including NK (Yoon et al., 2005; Yoon et al., 2007). The response to cord blood serum appears
878 to be related to the severity of NK with mild to moderate (stages 1 and 2) lesions responding
879 quicker than severe (stage 3) defects. (Erdem et al., 2014). ~~Human cord blood serum eye drops~~
880 has greater concentrations of nerve growth factor and other mediators but s~~However, small~~
881 volumes obtained from the placenta, limits general use. Platelet-rich plasma (PRP) is abundant in
882 growth factors that promotes ocular surface regeneration (Hartwig et al., 2004; Hartwig et al.,
883 2005). Despite the differences in methods used to prepare this product for clinical use, studies
884 indicate that PRP gives better results than autologous serum and can lead to healing of persistent
885 epithelial defects where autologous serum drops have failed. They also have a good safety
886 profile (Kim et al., 2012; López-Plandolit et al., 2010; Soni and Jeng, 2016). Its content of

887 [biologically active proteins, growth factors, and biomaterial scaffolds make PRP a therapeutic](#)
888 [agent promoting ocular surface wound healing and regeneration \(Anitua et al., 2016\). PRP has](#)
889 [also demonstrated efficacy, as a monotherapy, in the management of post laser refractive surgery](#)
890 [‘ocular surface syndrome’ \(Alio et al., 2017\).](#)

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895 *10.1.3 Prevent stromal tissue loss*

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

905

906 *10.1.4. Biological medical products*

907 Drugs given systemically targeting mediators that fuel inflammation or delay wound
908 healing have gathered momentum over the past two decades, although very few biologics have
909 been licensed for ocular use. Topically administered biologics or biosimilars provide an
910 attractive area for drug development. Murine NGF (Bonini et al., 2000; Lambiase et al., 1998),
911 Substance P and Insulin-like growth factor (Yamada et al., 2008) were the first mediators to
912 show encouraging results (Fig. 132). A number of novel treatment modalities have emerged
913 through clinical trials and are available for clinical use. These include recombinant human NGF
914 (rhNGF, cenegermin (betaNGF) (European Medicines Agency, 2017), lifitegrast 5%
915 (lymphocyte function-associated antigen-1 (LFA-1) antagonist) (Perez et al., 2016),
916 ReGeneraTing Agent [RGTA] – matrix therapy agent, Cacicol20, Thymosin beta 4, Conenzyme
917 Q10, Substance P, Netrin-1 (class of proteins involved in axon guidance and cell migration) and
918 Nexagon® (an antisense oligonucleotide that downregulates expression of the gap junction
919 protein Cx43, which is increased in pathological conditions with persistent epithelial defects)
920 (Guerra et al., 2017). Many of these agents promote healing in a generic way by combating
921 inflammation (lifitegrast) or rejuvenating the stroma by providing binding sites for growth
922 factors to promote healing (RGTA). NGF specifically targets the deficit in NK by replacing the
923 nerve growth factor and promoting epithelial healing and nerve health.

924 Cenegermin is a recombinant form of human nerve growth factor (rhNGF) produced in
925 *Escherichia coli* as a pro-peptide, which is later cleaved to mature NGF. The molecule is
926 identical to human NGF (European Medicines Agency, 2017). The European Medicines Agency
927 recently (July 2017) granted Cenegermin 20 µg/ml (Oxervate®) full marketing authorization for
928 the treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) NK in adult

929 patients by, making it the first approved medical treatment for this specific indication. The
 930 efficacy and safety of cenegermin were evaluated in two independent, multicentre, randomised,
 931 double-masked, vehicle-controlled clinical studies (NGF0212 and NGF0214) in patients with
 932 moderate or severe NK refractory to non-surgical treatments. In both studies patients received
 933 cenegermin or vehicle 6 times daily in the affected eye(s) for 8 weeks, and were followed-up.
 934 Study NGF0212 (NCT01756456) conducted in Europe, enrolled a total of 174 patients (mean
 935 age 61±16 years); 156 patients were assessed independently for efficacy, comparing two
 936 different dosages of the medicinal product with 20 and 10 µg/ml cenegermin to vehicle (52
 937 patients per arm) (Mantelli et al., 2017; Sinigaglia and NGF Study Group, 2014). Study
 938 NGF0214 (NCT02227147), run in US, enrolled 48 patients (mean age 65±14 years) treated with
 939 cenegermin 20 µg/ml or vehicle (24 patients per arm) (Chao et al., 2017). ~~A summary of the~~
 940 ~~results of the two studies in relation to e~~Complete corneal healing of the persistent epithelial
 941 defect or corneal ulcer (the ~~key efficacy primary~~ endpoint, defined as the greatest diameter of
 942 corneal fluorescein staining <0.5 mm) after 4 and 8 weeks of treatment for patients who received
 943 cenegermin 20 µg/ml or vehicle ~~was compared, is given in table 5. There was a statistically~~
 944 ~~significant improvement to complete healing of the cornea at 4 and 8 weeks of treatment (58.0%~~
 945 ~~and 74.0% respectively) compared to vehicle (19.6% and 43.1% respectively) (p <0.001 and~~
 946 ~~<0.002) in study NGF 0212. In study NGF 0214 the difference was statistically significant at 8~~
 947 ~~weeks of treatment (69.6% with cenegermin and 29.2% with vehicle, p<0.006).~~

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949 10.2 Non-surgical interventions

950 10.2.1 Eyelid closure

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

957 Placing an adhesive tape (transpore) across the closed eyelids is a simple intervention to
958 keep the lids closed. ~~This is routinely employed by anesthetists to prevent exposure of the cornea~~
959 ~~during surgery under general anesthesia. When used in the context of non-healing epithelial~~
960 ~~defects of the cornea.~~ The limitation of taping is that if repeated removal and (re) application is
961 required to instill eye drops or examine the eye, the delicate skin of the lids can excoriate and be
962 bruised. The 'Apache patch' and invention of Mr B Donaldson of Aberdeen, Scotland, is a clever
963 eye patch device which has two components, a 'T' shaped part for the upper lid with adhesive
964 backing to the horizontal limb and a small rectangular part, with adhesive backing, for the lower
965 lid. ~~The lower end of the vertical limb can be affixed to the part on the lower lid with Velcro.~~
966 ~~This allows the~~ Velcro components ~~cant~~ be separated and reattached without removing the
967 device from the lids. The Frost suture (can be considered a surgical intervention), which
968 involves the placement of suture (4 '0 silk) horizontally through the skin of the upper (or lower)
969 lid close to the lash line, serves a similar purpose. ~~The suture is cut to desired length and the two~~
970 ~~ends tied. The suture can then be used as a 'handle' to pull the upper lid down (or the lower lid~~
971 ~~up) and close the eye and keep it closed by fixing the suture to the cheek (or forehead) with~~
972 ~~adhesive plaster.~~ Use of an eye pad with tape or bandage provides complete closure with some
973 pressure. Usually two pads are used, one folded in half and applied on the closed lids to fill the

974 space of the anterior orbit, and the other placed on this and attached with a couple of strips of
975 tape running from the forehead to cheek or held in place with an eye bandage. These are useful
976 when closure for the whole day is required and not when the eye has to be opened frequently
977 during the day. The drawback of needing to undo the patch or eyelid tape every time an eye drop
978 is due has to be administered, makes them impractical. Importantly, care has to be taken to
979 ensure that the lids do not open under the patch as this would allow the patch to rub against the
980 cornea and aggravate the condition.

981 Botulinum toxin induced ptosis is an exception to the above. It provides a safe, rapid and
982 effective means of closing the eye whilst still allowing for easy access to examine or instill
983 eyedrops (Fig. 143). An injection of botulinum toxin is administered at the upper border of the
984 superior tarsal plate or over the belly of the levator palperae superioris muscle and induces
985 paralysis thereof causing the upper lid to droop and completely cover the cornea. The effect
986 typically lasts 6 to 12 weeks. The number of units, dilution and volume to be injected varies
987 according to form of botulinum toxin used. Another consideration is that the effect of the toxin is
988 not immediate and can take several hours or a couple of days. Hence repeat injections should be
989 avoided in the same week. In cases where only partial closure occurs, lagophthalmos may result
990 in exposure and compound the problem and a further injection of botulinum toxin may be
991 required (Kirkness et al., 1988; Schilimow and Wiechens, 2016).

992 *10.2.2 Therapeutic Contact Lenses*

993 Therapeutic contact lenses are fitted to maintain the integrity of the ocular surface tissues
994 where improvement of vision is secondary benefit. Commonly used biomaterials are silicone
995 hydrogels and rigid gas permeable, that are fitted as corneal, limbal, semi-scleral or scleral

996 lenses. Rigid gas permeable scleral contact lenses are vaulted away from the cornea and
997 supported by the anterior sclera. The design creates a reservoir between the lens and cornea that
998 captures therapeutic substances and increases retention time of therapeutic agents and lubricants
999 on the ocular surface. One such device is the prosthetic replacement of the ocular surface
1000 ecosystem (PROSE) device. The use of contact lenses in the context of NK must be accompanied
1001 by extreme vigilance. The reduction of sensation reduces 'alarm' signals for infection. If used,
1002 prophylactic use of topical non-preserved antibiotics is advised (Baenninger et al., 2014).

1003 Innovative devices using a combination of amniotic membrane and a contact lens are in
1004 vogue. The OmniLenz® and Prokera™ (see later) are examples. OmniLenz® consists of a soft
1005 contact lens lined with a circular disc of vacuum dried amniotic membrane and is applied
1006 directly on the corneal surface. The amniotic membrane rapidly hydrates and provides a soft
1007 biological cover to the defect. It is suggested that the release of molecules from the amniotic
1008 membrane favour healing. Evidence from clinical trials is lacking though anecdotal experience
1009 with single cases has shown a beneficial effect.

1010 *10.2.3 Punctal Occlusion*

1011 ~~The tear film contains many factors that aid in corneal epithelial and stromal healing.~~
1012 ~~These include growth factors, such as EGF, TGF, TGF- β , bFGF, HGF, and vitamins A and C,~~
1013 ~~and fibronectin. Increasing the amount of tears in the eye can aid in the healing of epithelial~~
1014 ~~defects and nonhealing ulcers. In addition, tears lubricate the surface of the eye, remove disease-~~
1015 ~~causing pathogens, and protect healing epithelium. Low volume of tears can be compensated for~~
1016 ~~either by adding artificial tears or decreasing tear egress from the palpebral fissure. Tear~~
1017 ~~substitutes do not contain the growth factors and vitamins present in natural tears. Punctal~~

1018 occlusion increases the retention of natural tears enhancing the healing process (Cohen, 1999;
1019 Guzey et al., 2001). Punctal occlusion can be performed by various methods (Baxter and
1020 Laibson, 2004). Punctal plugs – these can be temporary or short acting collagen plugs, or
1021 permanent punctal or canalicular plugs. The punctae can be also be permanently occluded with
1022 thermal cautery. Other methods of occluding the puncta, include argon laser, suturing of the
1023 punctum, or canalicular ligation (DeMartelaere et al., 2006; Hutnik and Probst, 1998; Liu and
1024 Sadhan, 2002).

1025 Punctal plugs can be associated with complications, such as pyogenic granulomas,
1026 bacterial colonization, extrusion, and local irritation (Kim et al., 2005; Sugita et al., 2001; Tai et
1027 al., 2002). Timing is critical as early insertion during active inflammation may lead to the
1028 accumulation and stagnation of tears loaded with pro-inflammatory cytokines and pro-
1029 inflammatory mediators gene expression on the ocular surface (Tong et al., 2016).

1030 *10.3 Surgical intervention*

1031 Surgery is often required in advanced disease refractory to medical management, mainly
1032 in moderate and severe NK (stages 2 and 3). Medical and surgical options are not mutually
1033 exclusive and are often combined.

1034 *10.3.1 Permanent punctal occlusion*

1035 Where temporary punctal occlusion has been successful in improving the tear reservoir,
1036 epiphora has not occurred and there is no prospect of a return of normal lacrimation then
1037 permanent punctal occlusion may be considered. This is particularly beneficial in patient likely
1038 to require lifelong punctal occlusion in whom punctal plugs can give rise complications stated

1039 above. Cauterisation of the vertical part of the canaliculus and punctum with heat is the standard
1040 method to achieve this.

1041 *10.3.2 Tarsorrhaphy*

1042 Tarsorrhaphy provides a more definitive closure of the eyelids by approximating the
1043 upper and lower lids. This is considered as the gold standard in NK treatment in several centres
1044 (Fig. 154). Closure of the lids protects the cornea from the environment, prevents epithelial
1045 damage by the friction caused by eyelid movement especially when the lid margins are irregular
1046 and keratinized (Cosar et al., 2001), conserves tear fluid and provides a reservoir of tears to keep
1047 the eye constantly moist and theoretically, the approximation of the vascular palpebral
1048 conjunctiva to the corneal surface affords additional unknown benefit. Tarsorrhaphy can be
1049 temporary or permanent, depending largely on the natural history of the underlying etiological
1050 condition (Allen and Malinovsky, 2003), partial (lateral, medial, central) or complete, depending
1051 on the severity of NK.

1052 Of the various techniques of performing a tarsorrhaphy, the most common is suturing the
1053 lids to each other over bolsters. This tarsorrhaphy reverses when the suture is removed and often
1054 the suture loosens after a few days or weeks. If the lid margins are denuded prior to suturing, the
1055 tarsorrhaphy is permanent, and if they are not, it is temporary. The advantage of (lateral)
1056 tarsorrhaphy over corneal patching is that the eye can be examined, the patient has vision, and
1057 the risk of infectious keratitis is reduced (Ali and Insler, 1986; Cosar et al., 2001; Panda et al.,
1058 1999). Tarsorrhaphy should be considered in all cases of persistent epithelial defects that fail to
1059 respond to medical treatment and/or non-surgical interventions (Tuli et al., 2007). If healing
1060 occurs, the tarsorrhaphy opening may be enlarged after a few weeks, but opening the
1061 tarsorrhaphy prematurely may results in a recurrence of corneal epithelial breakdown, especially

1062 if total corneal anaesthesia persists.

1063 *10.3.3 Debridement*

1064 At times, the leading edges of the healing epithelium may thicken and become rolled or
1065 heaped, impeding migration across the defect. In such cases, the epithelium at the edges of the
1066 defect may be removed, effectively enlarging the defect. This triggers the healing response in the
1067 surrounding epithelium promoting migration to close the defect (Katzman and Jeng, 2014).

1068 *10.3.4 Amniotic Membrane Transplantation (AMT)*

1069 The amniotic membrane (AM) is a versatile tissue that has caught the imagination of
1070 ophthalmologists and has been used for a wide variety of indications across several ophthalmic
1071 subspecialties. Its efficacy has been adequately demonstrated (Azura-Blanco et al., 1999; Dua
1072 et al., 2004; Gomes et al., 2005), but equally it is at times used “as something to do rather than
1073 something that does” (*hsd*) (Clare et al., 2012; Joseph et al., 2001; Rahman et al., 2009). AM can
1074 be used as a graft (inlay) or a patch (onlay) (Bonini et al., 2003). When the AM is applied such
1075 that epithelium migrates on the membrane and the amnion becomes incorporated in the cornea, it
1076 is termed a ‘graft’ (Fig. 165). Conversely if the healing epithelium migrates under the AM and
1077 the amnion later falls off or is removed, it is termed a ‘patch’. At times two membranes can be
1078 used one as a graft and the other as a patch over the graft. AMT can be combined with
1079 tarsorrhaphy but individually they have shown efficacy in the treatment of refractory
1080 neurotrophic corneal ulcers (Khokhar et al., 2005). Multiple pieces of AM cut to fit the shape of
1081 the defect can be stacked and finally covered by a graft or patch (Prabhasawat et al., 2001). The
1082 amnion allows keratocytes to migrate in the AM stroma and lay down collagen/scar tissue, which
1083 helps build the tissue at the site of melt. Multiple layers of amniotic membrane can integrate into

1084 the corneal stroma with resulting increase in corneal thickness; however keratocyte-mediated
1085 wound healing and remodeling of the incorporated amniotic tissue induces progressive
1086 contraction and changes in tissue transparency (Nubile et al., 2011). In the context of NK it has
1087 proven efficacy and is usually used in severe NK (stage 3) but has been used in mild and
1088 moderate NK (stages 1 and 2). (Gris et al., 1999).

1089 Fresh, cryopreserved (Amniograft), freeze dried (Ambio dry) and vacuum dried
1090 (Omnigen) amnion are available and all have demonstrated efficacy to a lesser or greater extent,
1091 with the latter two offering advantages of ease of storage and transportation at room temperature.
1092 All human derived tissue carries a serious risk of transmission of infectious disease. Though
1093 'fresh' amnion is still used in some parts of the world, the practice does not allow sufficient time
1094 for a thorough testing for microbial contamination. Where serological testing is performed, the
1095 donor is tested at the time of donation and the tissue quarantined for 6 months, when a repeat test
1096 is performed. The material is released for clinical use only when both tests are negative. With
1097 PCR testing on tissue samples, it is possible to release tissue within a week, when theoretically it
1098 would be classed as 'fresh'. The membranes can be applied to the defect with tissue glue (fibrin
1099 glue, Tisseel®) or sutures. Lyophilized AM has been shown to have lower concentrations of
1100 proteins/growth factors (Rodriguez-Ares et al., 2009). AM has many features that make it
1101 extremely useful for the prevention and treatment of corneal ulceration (Tseng et al., 2004). Its
1102 basement membrane is composed of collagen types IV and VII, laminin 1 and 5 and fibronectin
1103 (Cooper et al., 2005). The laminin and fibronectin assist in epithelial cell adhesion and are
1104 therefore useful in treatment of PEDs (Cameron et al., 1988; Nakagawa et al., 1990). The stroma
1105 also contains multiple growth factors (EGF, TGF- α , KGF, HGF, bFGF, TGF- β 1, - β 2), anti-
1106 angiogenic factors (thrombospondin-1 and collagen VIII), TIMPs (1,2,3 and 4) and anti-

1107 inflammatory factors (IL-1 receptor inhibitor and IL-10) that may help in the resolution of ulcers
1108 and decrease scarring (Koizumi et al., 2000). The major use of AMT in corneal pathology is in
1109 the management of neurotrophic ulcers (severe NK) and PEDs. Kruse et al. evaluated multilayer
1110 AMT in neurotrophic ulcers that had failed after at least 4 weeks of conventional therapy with
1111 lubrication, patching, or bandage contact lenses. They found that all the ulcers resolved at 4-5
1112 weeks following AMT, but the surface layer of AMT disappeared faster than the ulcer healed,
1113 and multiple layers were necessary to achieve resolution of the ulcer (Kruse et al., 1999). Chen et
1114 al. performed a similar study on longstanding neurotrophic ulcers of various etiologies that had
1115 failed conventional therapy (Chen et al., 2000). They found that 76% of the eyes had rapid
1116 epithelial healing within 16 days. However, more than half of their patients also needed
1117 adjunctive therapy with tarsorrhaphy, bandage contact lens, or bandage amniotic membrane,
1118 reiterating that single layer AMT may not be sufficient for severe neurotrophic ulcers.

1119 Prokera™, which consists of an amniotic membrane clipped into a dual PMMA ring set,
1120 has been used for the treatment of chronic ulcers. The advantage of this device is that it does not
1121 need sutures for placement and can be easily slipped into the eye like a large scleral contact lens
1122 (Suri et al., 2013). Other commercially available are Amnion (Bio-Tissue, Inc., Miami, FL),
1123 fresh frozen, and Ambiodry2 (IOP Ophthalmics, Costa Mesa, CA), freeze-dried. A suspension of
1124 homogenized amniotic membrane in BSS has been used topically in patients with ulcers
1125 refractory to conventional therapy. Healing of all ulcers occurred by 28 days following
1126 institution of therapy (Bonci et al., 2005).

1127 *10.3.5 Tissue adhesives*

1128 Tissue adhesives have been used for the closure of corneal defects and perforations for
1129 many years (Bloomfield et al., 1963; Refojo et al., 1968). In the presence of a small perforation

1130 (less than 3mm) the application of tissue adhesive on the lesion, followed by the application of a
1131 soft bandage contact lens or AMT is the procedure of choice. Larger defects require a
1132 conjunctival flap or lamellar keratoplasty (Mantelli et al., 2015). Two basic types of adhesives
1133 are used in ophthalmology, synthetic (cyanoacrylate) and biologic (fibrin glue) (Bhatia, 2006).
1134 Cyanoacrylate polymerizes rapidly in the presence of water (tissue fluid) and also releases
1135 formaldehyde during polymerisation. Formaldehyde contributes to its antibacterial activity
1136 against most gram-positive organisms (de Almeida Manzano et al., 2006; Diaz-Valle et al.,
1137 2003). It forms a rigid, impermeable plaque on the surface of the eye, which needs to be covered
1138 with a bandage contact lens to offer protection and avoid pain and trauma to the upper lid. The
1139 contact lens also prevents the glue being dislodged by lid movement. It remains in the eye long-
1140 term, as it is not biodegradable. Multiple perforations or a single perforation that continues to
1141 leak from the edge of the first patch of glue application, requires one or more overlapping further
1142 applications (Fig. 176). When there is a perforation with iris prolapse, the double drape
1143 technique can be used. The prolapsed iris is covered with a circular disc of plastic drape without
1144 glue and this in turn is covered with a larger disc of plastic drape with cyanoacrylate glue. The
1145 second disc adheres to the corneal tissue around the first disc, which prevents the glue from
1146 directly adhering to the iris (Gandhewar et al., 2013). The epithelium usually grows under the
1147 glue and healing of the defect usually dislodges the glue.

1148 Fibrin glue polymerizes relatively slowly and is therefore less effective in frank
1149 perforations with a brisk leak. Fibrin is also rapidly degraded, but the addition of aprotinin
1150 (antifibrinolytic agent) delays lysis for up to 10-14 days. Placement of a bandage contact lens
1151 after fibrin glue application also helps to retard degradation possibly by preventing access of
1152 polymorphonuclear cells and their proteases. The fibrin scaffold allows migration of

1153 keratocytes/fibroblasts and myofibroblasts, which in turn lay down collagen and help close the
1154 defect. Fibrin glue carries a theoretical risk of disease transmission as it is derived from pooled
1155 plasma. The advantage of fibrin glue is the higher comfort level and the absence of toxic
1156 metabolites (Sharma et al., 2003).

1157 *10.3.6 Conjunctival Flap*

1158 A conjunctival flap may be indicated to prevent progression of the epithelial defect to
1159 perforation (Pushker et al., 2001). Total conjunctival flaps are more useful in patients with severe
1160 stromal damage and poor visual prognosis. Partial or bridge flaps may be used for small or
1161 peripheral ulcers. Conjunctival flaps have some disadvantages that include corneal perforation
1162 under the flap, flap retraction, poor reversibility and the need for an operating room. Despite the
1163 disadvantages, conjunctival flaps can be helpful because they halt the inflammatory process, and
1164 eliminate need for frequent instillation of medication.

1165 The procedure of using a flap of bulbar conjunctiva to cover nonhealing corneal
1166 ulceration was first described by Trygve Gundersen in 1958 (Gundersen, 1958). Gundersen's
1167 flaps were frequently used for this purpose, but they fell into disfavor because they were difficult
1168 to fashion, especially in patients who had undergone multiple ocular surgeries, and they
1169 sometimes retracted. Other modalities for treating these conditions, such as disposable
1170 therapeutic contact lenses and AMT, were also much easier to use. However, in selected cases,
1171 conjunctival flaps may be superior, as they can replace an unhealthy stromal bed with healthy
1172 basal tissue on which epithelium can grow. In addition, they provide vessels and a blood supply
1173 to diseased cornea. These vessels aid in healing of resistant infections and provide serum-based
1174 growth factors. Vascularized structures are also very resistant to ulceration and perforation
1175 (Conn et al., 1980). The original flap procedure involved performing a 360° peritomy, debriding

1176 the corneal epithelium completely, and mobilizing the superior conjunctiva to cover the entire
1177 cornea. Various modifications have been made to that original technique in an attempt to
1178 decrease complications and increase the success rate, especially the use of partial pedicle grafts
1179 (Alino et al., 1998; Khodadoust and Quinter, 2003; Sandinha et al., 2006). It is generally
1180 accepted that conjunctival flaps need to retain a pedicle to ensure continued supply of blood.
1181 This makes mobilization and advancement of conjunctiva on to the cornea difficult in cases
1182 where the underlying pathology for NK, such as chemical burns, affects the conjunctiva also.
1183 Dua et al. proposed use of a free autologous conjunctival graft from the opposite eye or from a
1184 distal surviving site in the same eye (Dua et al., 2012). The free graft covers the affected cornea
1185 in whole or part; the peripheral margin of the graft or at least part of it and the edge(s) sutured to
1186 viable tissue (with a blood supply intact). This allows blood vessels to connect with the vessels
1187 and in the graft that carry blood to the affected cornea, helping it heal. Other methods that have
1188 been tried for grafts to maintain globe integrity include buccal mucous membrane grafts, split
1189 thickness dermal grafts, and tenon's capsule grafts (Ma'luf and Awwad, 2005; Mauriello et al.,
1190 1988; Reim et al., 1992).

1191 *10.3.7 Corneal transplants*

1192 When all other options have failed to heal progressive corneal ulceration, a tectonic
1193 corneal transplant is often the last resort. Corneal transplants are preferably done as a planned
1194 procedure after the active ulceration has resolved and all inflammation has settled, though often
1195 it has to be performed in cases with impending perforation of perforated corneas. Risk of
1196 rejection and failure is greater with 'hot grafts' and grafts performed in background of NK are at
1197 high risk of failure (Jonas et al., 2001). However, tectonic grafts that are performed to preserve
1198 the structural integrity of the cornea have some advantages over other treatment modalities. The

1199 vision is often better than that achieved with glue, amniotic membranes, or conjunctival flaps if
1200 the visual axis is involved (Killingsworth et al., 1993; Vanathi et al., 2002). Corneal grafts may
1201 be lamellar or penetrating. The advantage of lamellar transplant is that the anterior chamber is
1202 not entered in corneas that have not perforated. However, they are often technically challenging
1203 and may have poor visual outcomes (Soong et al., 2000). Jonas et al. compared the outcomes of
1204 penetrating keratoplasty in patients with corneal ulcers and patients with corneal scars from
1205 healed corneal ulcers (Jonas et al., 2001). They found that the visual outcomes were poorer and
1206 there were more episodes of rejection and loose sutures in the tectonic grafts. However, the
1207 visual outcomes were much better than with the lamellar transplants. Elective corneal
1208 transplantation for visual rehabilitation in patients with NK carries a high risk of failure. These
1209 patients have poor epithelial wound healing and are prone to inflammation. Both of these factors
1210 significantly increase the risk of melting, perforation and rejection.

1211 A conjunctival flap is recommended when descemetocoele or perforation recurs despite
1212 previous corneal transplantation (Vasseneix et al., 2006). The Boston keratoprosthesis
1213 implantation has emerged as an effective modality for visual rehabilitation in such patients
1214 (Katzman and Jeng, 2014; Pavan-Langston and Dohlman, 2008).

1215 *10.3.8 Direct neurotisation*

1216 Direct neurotization of the cornea using the contralateral supraorbital and supratrochlear
1217 branches of the ophthalmic division of the trigeminal nerve has been performed for restoring the
1218 corneal sensitivity in patients with unilateral facial palsy and anesthetic cornea. Terzis et al
1219 (Terzis et al., 2009) described a novel surgical procedure in which donor nerve branches are
1220 inserted at the contralateral anesthetic corneal limbus for sensory neurotization. Use of the sural

1221 nerve for this purpose has also been described (Bains et al., 2015; Elbaz et al., 2014). This
 1222 surgical technique, although difficult to perform, preserves ocular anatomy and cosmesis and
 1223 restores function. A step-ladder approach to the use of medical, non-surgical and surgical
 1224 interventions in the management of NK is given in table 3.

1225

1226 **11. Future Directions**

1227 Testing corneal sensitivity is key to recognition and diagnosis of NK. Corneal sensitivity
 1228 testing is not routinely undertaken in clinical practice and is inadequately performed with the
 1229 help of a cotton ~~w~~hisip or tissue paper rolled to a fine tapering end with bare fingers (unsterile).
 1230 This, at best gives a qualitative estimate of the central cornea as it is usually performed in the
 1231 central cornea ignoring the four quadrants. The Cochet Bonnet aesthesiometer gives a reasonable
 1232 quantitative estimate of corneal sensitivity. Its nylon thread is re-usable, usually cleaned with a
 1233 sugical wipe but is not sterile. The Belmonte aesthesiometer is a non-contact device; ~~more~~
 1234 ~~sophisticated and accurate~~ but ~~unwieldy and~~ for practical reasons is not widely used. As a result,
 1235 NK remains an under-diagnosed condition. There is a strong need for a method or device that is
 1236 clinically practical and easy to use in busy clinics, yet providing a quantitative (or semi
 1237 quantitative) assesment. ~~Research is underway to produce a standardised clinical tool that is~~
 1238 ~~sterile, disposable, easy to use and give semi-quantitative assessment of corneal sensitivity. Such~~
 1239 ~~a device would be very desirable and should be available for trial in the next year or two.~~

1240 Corneal hyperaesthesia is recognised as a feature of corneal pathology and may also be part
 1241 of the process that eventually culminates in hypoaesthesia and complete loss of sensation. This can
 1242 be associated with increased firing of existing nerves or abberant regeneration of nerves. There is

1243 no method available to test increased sensitivity. In future, as understanding of hyperaesthesia
1244 and methods of assessing it will become important.

1245 The pathophysiology of NK is ill-understood and often compounded by the pathophysiology
1246 of the causative underlying condition or agent. This makes for inadequate staging or grading of
1247 the condition and treatment strategies are difficult to target towards specific factors in the
1248 etiopathogenesis. In addition, interactions between disease processes and iatrogenic interventions
1249 make diagnosis and treatments even more difficult; therapeutic protocols lack standardization
1250 and duration of treatment remains unclear as recurrences may occur if treatment is tapered too
1251 early or in cases where the underlying condition cannot be cured. ~~Lack of functional innervation
1252 and consequent deprivation of trophic environment increases risk of delayed or poor post
1253 surgical wound healing, limiting surgical options for restoration of vision, in corneas affected by
1254 NK. Nevertheless, an Nevertheless,~~ affection of the sensory nerves of the cornea is common to
1255 all stages/grades of NK. ~~Until recently and~~ there ~~was~~ no drug that addresses this factor. The
1256 ~~recent~~ advent of NGF with proven clinical efficacy through clinical trials offers a lot of promise.
1257 ~~and o~~Other topically administered products that promote ~~nerve health and growth~~epithelial
1258 healing, are in the pipeline. as therapeutic agents specifically aiming to restore effects of nerve
1259 ~~damage, offer promise.~~ These products should become available for clinical use in the near
1260 future leading to post marketing clinical trials, which in turn will establish the true potential of
1261 the products.

1262 Surgical neurotisation of the cornea with nerve grafts is an elaborate procedure, still in its
1263 infancy but with exciting and promising possibilities. The foreseeable future will see
1264 improvement in surgical techniques and improved outcomes making the surgery available to
1265 more patients by a greater number of trained surgeons. High resolution in vivo confocal

1266 microscopy imaging of the cornea following neurotisation or treatment with rhNGF or Netrin-
1267 I should allow direct visualisation of the new nerves sprouting from the transplanted trunks.

1268 Patients with NK manifest local (eye specific) symptoms, general health and social and
1269 psychological problems. Due to duration of the disease, its potential visual impact and the burden
1270 of the range of treatment options, quality of life can be severely affected by NK. There is a lack
1271 of a specific NK related tool to accurately assess quality of life (QoL) in these individuals. The
1272 main objectives of future therapies will thus be to tackle all these complex issues to heal the
1273 cornea, to prevent or reverse vision impairment and improve quality of life of NK patients.

1274

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1278

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1280

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1976 15. Legends for Figures

1977

1978 **Fig. 1.** [Diagram illustrating the afferent sensory pathway from the cornea and conjunctiva to the
 1979 trigeminal ganglion and the efferent sympathetic and parasympathetic nerve pathways. Adapted
 1980 and modified from Stern 2013.](#)

1981

1982 **Fig. 2.** Effect of benzalkonium chloride (BAK) on cultured corneal epithelial cells. H0 (hour
 1983 zero) the vertical gap created by the ‘scratch’ in a confluent layer of corneal epithelial cells is
 1984 visible. H8 (hour 8) the gap is fully closed to establish a confluent sheet again compared to the
 1985 BAK group where the defect persists.

1986

1987 | **Fig. 23.** Sequence of events leading to epithelial disturbance, epithelial loss and stromal lysis as
1988 neurotrophic keratopathy progresses through different stages/grades of severity.

1989

1990 | **Fig. 34.** Grades/stages of neurotrophic keratopathy (NK). (a) Slit lamp broad beam illumination
1991 showing central, confluent punctate lesions giving the central cornea a dull lack-lustre
1992 appearance following photorefractive keratectomy (ocular surface syndrome) (OSN). (b) The
1993 same cornea stained with fluorescein showing that most of the lesions take up stain highlighting
1994 the epitheliopathy. (c) OSN following laser in-situ keratomileusis. Fluorescein stain of the cornea
1995 shows coarse, centrally located, almost confluent 'keratitis'. (a – c represent grade/stage 1 NK).
1996 (d) The central cornea shows a diffuse haze with a large epithelial defect. (e) The central cornea
1997 is hazy with an epithelial defect with superficial and deep stromal vessels. (f) There is a large
1998 corneal epithelial defect with a relatively clear stroma. Images d-f represent grade/stage 2 NK.
1999 The white, rolled margins of the defects is a typical sign of a non-healing epithelial defect. (g)
2000 Slit view showing stromal melting to mid depth in a case of herpes zoster keratitis. (h) Deep
2001 stromal ulceration in a child with congenital anaesthetic cornea. (i) A large corneal ulcer in a
2002 case of treated acanthamoeba keratitis. Inset illustrated the stromal roughening, over which the
2003 epithelium did not migrate. Note the absence of corneal vascularization. Images g to i represent
2004 grade/stage 3 NK.

2005

2006 | **Fig. 54.** Complications of neurotrophic keratopathy (NK). (a) Three months following a
2007 chemical burn the eye shows limbal ischaemia in the inferior half with a large hypopyon. (b) The
2008 same eye as in (a) stained with fluorescein. A large non healing epithelial defect is seen. Corneal

2009 sensation was absent in the lower half. (c) NK with superadded bacterial infection, stromal
2010 melting and ectasia. (d) Same eye as in (c) showing that the area of epithelial defect, stained with
2011 fluorescein, corresponds to the stromal involvement. (e) NK in a penetrating corneal graft in an
2012 aphakic eye, showing a 'silent' central perforation with prolapse of the vitreous. (f) NK with
2013 Descemetocoele and perforation showing aqueous leakage (Sidel positive). The corneas in (e) and
2014 (f) developed perforation in the absence of infection.

2015

2016 | **Fig. 56.** Right eye of a patient with mild/stage 1 neurotrophic keratopathy (NK). (a) The lower
2017 half of the cornea shows multiple, almost confluent, punctate erosions stained with fluorescein.
2018 The upper half has a normal tear film. Inset shows the white appearance of the epitheliopathy.
2019 Cochet-Bonnet aesthesiometry measured 60mm in the upper half and 15mm in the lower half.
2020 (b) Higher magnification broad beam slit lamp illumination shows the white lesion on the right
2021 hand side of the beam and 'vesicular' appearance or intra-epithelial microcysts on the left side of
2022 the beam. Intraepithelial vesicular lesions are part of the epitheliopathy of NK. Superficial
2023 lesions rupture and stain with fluorescein.

2024 | **Fig. 67.** Left eye of the same patient as in figure 5. (a) The patient underwent penetrating
2025 keratoplasty for herpes simplex virus keratitis related scars. Post-operatively she presented with
2026 moderate/grade 2 neurotrophic keratopathy (NK). An epithelial defect with underlying and
2027 surrounding stromal haze is seen. (b) On fluorescein staining the ulcer is delineated. The
2028 surrounding epithelium shows coarse punctate keratitis. (c) NK progressed to severe grade/ stage
2029 3. Various treatment options were tried including an amniotic membrane patch. The ulcer healed
2030 but left a scarred vascularized cornea, shown a year later. (d) Two years post-graft the corneal
2031 graft has failed with a dense scar and further vascularization. Corneal sensations were absent.

2032 The case illustrates that with conventional therapeutic interventions, 'successful' healing of
2033 severe NK can be associated with severely compromised vision.

2034

2035 | **Fig. 78.** Progression of neurotropic keratopathy (NK). (a) Following successful treatment of
2036 | acanthamoeba keratitis, there was marked reduction of corneal sensation (Cochet-Bonnet 5 mm)
2037 | with moderate/stage 2 NK. (b) NK progressed with stromal lysis and (c) perforation, despite
2038 | treatment.

2039

2040 | **Fig. 89.** Diagnostic algorithm for neurotrophic keratopathy.

2041

2042 | **Fig. 910.** Testing corneal sensation. (a) Testing corneal sensation with a wisp of cotton. This is a
2043 | qualitative test and easy to perform at the bedside. However, the cotton is usually not sterile or
2044 | even if so, is drawn into a thin wisp with 'un-sterile' fingers. (b) testing corneal sensation with
2045 | the Cochet-Bonnet aesthesiometer. The fine nylon thread is sterilised by wiping it with an
2046 | alcohol swab. (c) The set up of the Belmonte aesthesiometer. A controlled jet of air is targeted on
2047 | the cornea and the patient's response is both observed and interrogated.

2048

2049 | **Fig. 1011.** (a) A case of severe neurotrophic keratopathy (NK) examined by in-vivo confocal
2050 | microscopy (IVCM). (b) IVCM of the ulcerated area appears as a dark hypo-reflective patch
2051 | with scattered fine hyper-reflective speck (possible inflammatory cells/reactive keratocytes) and
2052 | surrounded by the hyper-reflective epithelial cells of the ulcer margin. (c) A large stromal nerve

2053 is seen as a hyper-reflective line in the deep stroma of the peripheral non-ulcerated area. (d) The
2054 stroma in the vicinity of the ulcer shows disorganization with reactive keratocytes. (e) The
2055 epithelium in the vicinity of the ulcer shows altered morphology and absence of the sub-basal
2056 plexus.

2057

2058 | **Fig. 412.** Anterior segment optical coherence tomography (ASOCT) in neurotrophic keratopathy
2059 (NK). (a) Severe NK with stromal melting. (b) ASOCT of the cornea illustrating the depth of the
2060 ulcer and its contour. The facet is filled with instilled tear drops. The density of the stroma
2061 (yellow red colour) is greater in the stroma around the ulcer. Anterior bowing of the posterior
2062 corneal layer can be appreciated indicating the start of a Descemetocoele.

2063

2064 | **Fig. 4213.** Response to treatment with recombinant nerve growth factor (NGF) eye drops. (a)
2065 Moderate grade/stage 2 neurotrophic keratopathy. (b) & (c) complete closure of the epithelial
2066 defect occurred with NGF drops instilled six times a day for a few weeks.

2067 | **Fig. 4314.** Beneficial effect of botulinum toxin induced ptosis in neurotrophic keratopathy (NK).
2068 (a) The cornea shows a large area of moderate grade/stage 2 NK. (b) Complete ptosis of the
2069 upper lid is achieved with botulinum toxin injection. (c) Healing of NK at 2 weeks and (d)
2070 complete healing at 4 weeks.

2071

2072 | **Fig. 4415.** Tarsorrhaphy, arguably the most effective surgical intervention in neurotrophic
2073 keratopathy (NK). (a) A cornea with unilateral chemical burn was treated with autologous limbal

2074 transplant. A persistent epithelial defect is seen (NK moderate grade/stage 2). (b) A lateral
 2075 tarsorrhaphy was performed. (c) Complete healing of NK is seen. (d), (e) and (f) are
 2076 corresponding fluorescein stained images showing the original epithelial defect, reduction in size
 2077 of the defect and complete healing respectively.

2078

2079 | **Fig. 4516.** Amniotic membrane transplant (AMT) in management of severe neurotrophic
 2080 keratopathy (NK). (a) Severe (stage 3) NK following trabeculectomy operation, which did not
 2081 respond to medical management. The central stroma is necrotic and surrounded by a gutter of
 2082 stromal lysis. (b) The ‘gutter’ is epithelialized but no epithelium has grown over the central
 2083 necrotic stroma (fluorescein stained). (c) An AMT (Omnigen 500 graft) with a running 10 ‘O
 2084 nylon suture was used to cover the defect after dissecting off the necrotic stroma.

2085

2086 | **Fig. 4617.** Corneal perforation managed by application of cyanoacrylate tissue adhesive. (a) A
 2087 case of bacterial corneal ulcer, medically treated with intensive antibiotics. The ulcer was
 2088 rendered sterile but remained as a persistent defect with reduced sensation, progressing to
 2089 perforation at two sites. Multiple patches of cyanoacrylate glue were required to seal the
 2090 perforations. (c) Two months later, the glue has been dislodged revealing a scarred and
 2091 vascularized cornea. The perforations have sealed and the anterior chamber is formed.

2092

2093

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

Non-Healing corneal epithelial defects
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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

<ul style="list-style-type: none"> • <i>Genetic</i> (Morishige et al., 2014) <ul style="list-style-type: none"> ○ Riley–Day syndrome (familial dysautonomia) ○ Goldenhar–Gorlin syndrome ○ Mobius syndrome ○ Familial corneal hypoaesthesia • <i>Systemic</i> <ul style="list-style-type: none"> ○ Diabetes mellitus. (Lockwood et al., 2006)
--

○ Leprosy
○ Vitamin A deficiency
○ Amyloidosis
○ Multiple sclerosis
• <i>Central nervous system</i>
○ 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
• <i>Ocular</i>
○ Post-herpes infections (herpes simplex and herpes zoster)
○ <u>Other infections e.g acanthamoeba with nerve damage related to keratoneuritis</u>
○ Chemical and physical burns
○ Abuse of topical anaesthetics
○ Drug toxicity (timolol, betaxolol, diclofenac sodium, sulphacetamide 30%)
○ Chronic ocular surface injury or inflammation
○ Ocular surgery <ul style="list-style-type: none"> ▪ 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)	<ul style="list-style-type: none"> • 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. 	<ul style="list-style-type: none"> • Improve epithelial quality and transparency. • Stabilise epithelium and avoid epithelial breakdown. • Prevent progression to Moderate grade (stage 2, persistent epithelial defect).
Moderate (stage 2)	<p><i>As per Stage 1 and:</i></p> <ul style="list-style-type: none"> • Prophylactic topical preservative-free antibiotics. • Prevention of melting with Citrate / tetracycline / macrolides (if stromal involvement is threatened) • Recombinant Human (rh)NGF (Cenergermin / 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. 	<ul style="list-style-type: none"> • Promote epithelial healing • Prevent the occurrence/recurrence of the epithelial breakdown • Prevent progression to Severe grade (stage 3, stromal lysis)
<i>As per Stage 2 and:</i>		

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).
- Promote corneal healing.
- Prevent further corneal stromal lysis and perforation.

In the event of perforation

- Cyanoacrylate tissue adhesive with therapeutic contact lens.
 - Fibrin glue.
 - Amniotic membrane graft or corneal grafts.
-

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

Table 4: Summary of ocular lubricants

Viscosity	Compound
Low	Hypromellose 0.3% Polyvinyl alcohol
Thin-medium	Carbomers Carmellose 0.5-1.0%
Thick-medium	Hyaluronates (0.1-0.4%) Trehalose 3%
High	Paraffin/white petroleum Retinoic acid ointment

Table 5. Shows efficacy of cenegermin 20 µg/ml compared to vehicle in the two studies.

		Study NGF0212		Study NGF 0214	
Results after 4 and 8 weeks of treatment		Week 4	Week 8	Week 4	Week 8
Complete corneal healing rate	OXERVATE	58.0 %	74.0 %	56.5 %	69.6 %
	vehicle	19.6 %	43.1 %	37.5 %	29.2 %
	(p value)	(0.001)	(0.002)	(0.191)	(0.006)

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