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Review

Clinical associations of corneal neuromas with ocular surface diseases

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From the Contents

Abstract

Corneal neuromas, also termed microneuromas, refer to microscopic, irregularly-shaped enlargements of terminal subbasal nerve endings at sites of nerve damage or injury. The formation of corneal neuromas results from damage to corneal nerves, such as following corneal pathology or corneal or intraocular surgeries. Initially, denervated areas of sensory nerve fibers become invaded by sprouts of intact sensory nerve fibers, and later injured axons regenerate and new sprouts called neuromas develop. In recent years, analysis of corneal nerve abnormalities including corneal neuromas which can be identified using *in vivo* confocal microscopy, a non-invasive imaging technique with microscopic resolution, has been used to evaluate corneal neuropathy and ocular surface dysfunction. Corneal neuromas have been shown to be associated with clinical symptoms of discomfort and dryness of eyes, and are a promising surrogate biomarker for ocular surface diseases, such as neuropathic corneal pain, dry eye disease, diabetic corneal neuropathy, neurotrophic keratopathy, Sjögren's syndrome, bullous keratopathy, post-refractive surgery, and others. In this review, we have summarized the current literature on the association between these ocular surface diseases and the presentation of corneal microneuromas, as well as elaborated on their pathogenesis, visualization via *in vivo* confocal microscopy, and utility in monitoring treatment efficacy. As current quantitative analysis on neuromas mainly relies on manual annotation and quantification, which is user-dependent and labor-intensive, future direction includes the development of artificial intelligence software to identify and quantify these potential imaging biomarkers in a more automated and sensitive manner, allowing it to be applied in clinical settings more efficiently. Combining imaging and molecular biomarkers may also help elucidate the associations between corneal neuromas and ocular surface diseases.

Key Words: cornea; corneal diseases; corneal nerve; corneal neuropathy; *in vivo* confocal microscopy; microneuroma; neuroma; ocular surface diseases

Introduction

Corneal neuromas, also termed microneuromas, are pathological indicators of corneal neuropathy (McCarty, 1998; Aggarwal et al., 2019) and ocular surface dysfunction (Giannaccare et al., 2017). They are microscopic, irregularly-shaped enlargements of terminal subbasal nerve endings and axonal sprouting at the site of nerve damage (Tiltman and Duffield, 1996), or represent stumps of severed nerves and abrupt nerve fiber endings (Aggarwal et al., 2015). The formation of corneal neuromas results from damage to corneal nerves, such as corneal pathology or corneal or intraocular surgeries. Initially, denervated areas of sensory nerve fibers become invaded by sprouts of intact nerve fibers, and later injured axons regenerate and neuromas develop, with the formation of new sprouts (Rózsa et al., 1983; Beuerman and Rózsa, 1984; Chang-Ling et al., 1990). At the sites of corneal nerve damage where neuromas form, neurons exhibit modified functionality and excitability, resulting in abnormal responses to external stimuli, causing spontaneous sensations of discomfort such as pain, dryness, and grittiness. Chronically, this may also give rise to allodynia or secondary hyperalgesia. On the other hand, the denervated corneal areas due to the severed nerve endings also paradoxically have reduced sensitivity to direct mechanical, chemical, or thermal stimuli (Belmonte et al., 2004a). Clinically, neuroma formation has been reported to be linked to post-surgical corneas such as post-photorefractive surgery (Yang et al., 2021) or ocular surface diseases, such as diabetic corneal neuropathy (Issar et al., 2020), neuropathic corneal pain (Aggarwal et al., 2015; Goyal and Hamrah, 2016; Dieckmann et al., 2017), dry eye disease (Guerrero-Moreno et al., 2021), neurotrophic keratopathy (Yavuz Saricay et al., 2021) and Sjögren's syndrome (Luzu et al., 2022). Hence there is a potential to use the changes in neuromas, such as the area or the density, as surrogate biomarkers to assess therapeutic efficacy (Chinnery et al., 2022), monitor the disease progression, or interpret the clinical efficacy of treatment. Visualization of neuromas can be performed via *in vivo* confocal microscopy (IVCM).

In this article, we provide a comprehensive review of current literature from publications centered around a biological structure termed "neuroma", "microneuroma", or other related terms, in the human cornea, in order to elucidate the underlying pathogenesis of neuromas and the association of neuromas with the clinical phenotypes of a variety of ocular diseases.

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Search Strategy and Selection Criteria

An electronic literature search on the PubMed database was performed in alignment with the primary research aim to elucidate the clinical association of neuromas and ocular diseases. The query performed in PubMed was a combination of keywords and MeSH terms, "(cornea [MeSH Terms] OR corneal nerve OR corneal epithelium OR corneal diseases[MeSH Terms] OR corneal diseases OR corneal diseases[Title/Abstract] OR corneal disease*) AND (neuroma [MeSH Terms] OR microneuroma)".

Databases were searched from inception to March 27, 2023, with no filters applied. From this search in the PubMed database, 123 references, published from 1964 to 2023, were retrieved. Thereafter, the references were checked for duplicates using the Covidence online screening tool (www.covidence.org), and no duplicates were found. Relevant articles selected included neuromas found in the corneal layer, and articles related to neuromas found outside the orbit, such as the eyelid, mucosal neuromas, or acoustic neuromas in the brain were excluded. Inclusion criteria consist of: all types of articles (clinical trial, meta-analysis, randomized controlled trial, review, and systematic review), articles with a date of publication in PubMed restricted to the most recent ten years, and the subject matter was related to humans. Exclusion criteria were: articles not written in the English language, or those without full text available. After considering inclusion and exclusion criteria, and screening the titles and abstracts for relevance, 16 references were shortlisted to be eligible for more intensive review, and the full-text version of all selected articles was subsequently assessed for eligibility. Supplementary relevant articles were also extracted from the bibliographies of the existing articles, to support the elaboration of the anatomy of the corneal nerve plexus, the pathogenesis of corneal neuromas, as well as visualization of corneal neuromas using IVCM. A total of 85 articles were included in the final manuscript. The selection process of references is detailed in **Figure 1**.

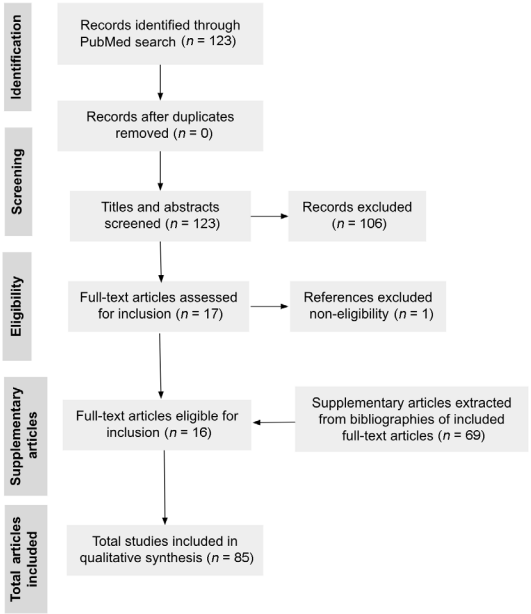

Figure 1 | **Flow diagram of the literature selection process for the present article.** A search was conducted on the online database PubMed for relevant articles describing the clinical association between corneal conditions and neuromas. Articles were included from inception till March 27, 2023 with the search query "(cornea [MeSH Terms] OR corneal nerve OR corneal epithelium OR corneal diseases[MeSH Terms] OR corneal diseases OR corneal diseases[Title/Abstract] OR corneal disease*) AND (neuroma [MeSH Terms] OR microneuroma)". After duplicate removal, titles and abstracts were screened based on inclusion and exclusion criteria, contributing to 16 full-text articles for closer examination. Relevant supplementary articles extracted from bibliographies of included full-text articles were also incorporated in the review. A total of 85 articles were included in the final manuscript.

The literature review on the association between corneal neuromas and ocular surface diseases is summarized in **Table 1**.

Anatomy and Function of the Corneal Nerve Plexus

The cornea, containing 7000 nerve fibers/mm^2 in the epithelium, has the highest nerve density in the human body (Luzu et al., 2022) and is sensitive to alterations in the local molecular factors and environmental influences (Müller et al., 2003). Corneal innervation mainly consists of somatic sensory innervation which originates from the ophthalmic division of the trigeminal nerve (Marfurt et al., 2010), which gives rise to the long and short ciliary nerves. Corneal nerves enter the cornea stroma radially, and travel anteriorly towards the corneal surface to pierce the Bowman's layer. The corneal nerve plexus, where many sensory nerve fibers meet, is located between the epithelial basement membrane and Bowman's layer (Marfurt et al., 2010). From the plexus, the nerve and branches spread through the epithelium of the cornea (Al-Aqaba et al., 2010).

Morphologically, ocular trigeminal neurons are mostly medium or small neurons with thin myelinated (A-delta) or unmyelinated (C) axons, which terminate peripherally into nociceptors. Functionally, corneal sensory nerves play crucial roles in the blink reflex, wound healing, and production of tears (Shaheen et al., 2014). Ocular surface neurons contain neuropeptides which are expressed as neurotransmitters, cytokines, neuromodulators, neurotrophins, growth factors, and ion channel proteins which regulate neuronal excitability (Felipe et al., 1999). Corneal neuromediators or neuropeptides are essential for corneal homeostasis including epithelial integrity, neuronal proliferation, plasticity, and apoptosis (Yang et al., 2021). Injury or degeneration of the corneal nerves thus leads to a neurotrophic ocular surface, presenting with decreased corneal sensitivity and in severe cases, to corneal ulceration (Al-Aqaba et al., 2019).

Pathogenesis of Corneal Neuromas

Corneal nerve fibers are susceptible to degeneration, inflammatory or toxic damage, partly due to the lack of direct vascular supply (Fernandes et al., 2021). When corneal nerves are severed, the denervated area is invaded initially by sprouts of intact nerve fibers, and subsequently injured axons begin to regenerate and neuromas form (Belmonte et al., 2004b). Neuromas thus represent sites of neuroregeneration where there is localized nerve damage following nerve injury (Kalangara et al., 2016), either due to systemic diseases that affect corneal nerves or ocular surgery. The initial nerve damage results in acute axonal injury and inflammation which can induce further nerve damage, Wallerian degeneration, and release of inflammatory mediators such as interleukin-1, substance P, and tumor necrosis factor-α, as well as cytokines which aid in removing cellular debris from degenerating axons, contributing to the repair process (Ross et al., 2020). At the site of axonal injury, the Schwann cell tube survives and directionally guides axons sprouting from the end of the proximal segment (Gaudet et al., 2011), with new sprouts appearing from neuromas (Rózsa et al., 1983; Beuerman and Rózsa, 1984; Chang-Ling et al., 1990). It was noted by Aggarwal et al. (2019) that "with axonal injury, the damaged axons seal the injured stump" where stumps are known as microneuromas, "and forms terminal bulbs with small fine branches in an attempt to regenerate". The failure to reestablish axonal continuity after disruption of abnormal axons causes neuromas to develop. These are a disorganized, tangled mass of Schwann cells, axons, and perineural fibroblasts, and can cause severe refractory neuropathic pain (Rosenthal et al., 2009; Kos et al., 2013; Cruzat et al., 2017; Aggarwal et al., 2019), and peripheral neuropathy (Koschmieder et al., 2020). Regeneration of neurons upregulates the expression of sodium channels, thus altering nerve activity. The neuroma nerve fibers have reduced sensitivity towards natural stimuli, manifesting as reduced corneal sensation (Belmonte et al., 2015). However, neuroma nerves are hyperexcitable and they are believed to undergo spontaneous and ectopic discharge, hence causing pain, hyperalgesia, allodynia (Belmonte et al., 2004b; Aggarwal et al., 2019), dryness, and grittiness (Moein et al., 2020; Stepp et al., 2020).

Visualization of Corneal Nerve and Neuromas via *In Vivo* Confocal Microscopy

IVCM is a real-time, non-invasive imaging technique with the microscopic resolution that allows direct visualization of corneal cellular ultrastructure layer by layer in both healthy and diseased corneas. It provides magnification up to 800-fold and high-resolution images, which are usually 384 × 384 pixels (400 µm × 400 µm horizontal × vertical), with 1–2 µm/pixel lateral resolution and 4 µm/pixel axial resolution (Teo et al., 2022a). IVCM is a reliable diagnostic and evaluation tool for nerve degeneration and regeneration (Liu et al., 2021), and has become the gold standard for assessing corneal nerve abnormalities, including decreased corneal nerve density or length, corneal nerve tortuosity, beading, and neuromas.

The IVCM modality involves first instilling topical anesthesia into the inferior fornix of the patient's eye, typically a drop of 0.5% proparacaine hydrochloride ophthalmic solution, then instructing patients to fixate on a light source with the contralateral eye to stabilize the scanning view. Laser scanning IVCM uses a 670 nm wavelength helium-neon diode laser source which does not pose any ocular safety hazard (Patel and McGhee, 2005), to scan regions of the cornea, and the scanned depth may vary depending on the study requirements.

Corneal neuromas on IVCM present as enlarged or engorged nerve endings (Cavalcanti et al., 2018), bulges, varicosities, tangles, and/or hyperreflective sites (Stepp et al., 2020). The most common description is "abrupt swelling(s) of injured nerve endings and neurite sprouting" (Cruzat et al., 2017), or irregularly shaped enlargement of terminal nerve endings with poorly defined margins and variable hyper-reflectivity (**Figure 2**; Moein et al., 2020). Ross et al. (2020) classified IVCM appearance of microneuromas into: (i) spindle: "hyper-reflective fusiform enlargement of a stromal nerve trunk without axonal sprouting", (ii) stump: "abrupt and swollen termination of the stromal nerves", and (iii) lateral: "localized hyper-reflective enlargements of a stromal nerve from which single or multiple tortuous nerves arose" (**Figure 2**).

Table 1 | **Studies reporting on the clinical association of corneal neuromas with ocular surface diseases**

Stuides	Study design	Study aims	Findings of studies
Diabetic corneal neuropathy			
Issar et al., 2020	Clinical trial	To determine the effects of time outside range and glucose variability on peripheral nerve structure and function in type 1 diabetes.	Short-term measures of poorer glucose control are associated with increased corneal microneuromas, nerve structural changes, and impaired nerve function.
Neuropathic corneal pain			
Ross et al., 2020	Prospective observational study	To describe *in vivo* confocal microscopy (IVCM) features of neuropathic corneal pain with absent clinical signs.	Microneuromas were identified by IVCM for all patients in the study group who presented with persistent ocular pain but with an unremarkable ocular surface on clinical examination. There were an average of 4.59 microneuromas per eye. There was significantly increased microneuromas in patients who responded to topical anesthesia, than those who did not respond ($P < 0.0001$). Spindle, lateral, and stump microneuromas were found in 74%, 44.4%, and 14.8% of eyes respectively. No microneuromas were found in healthy controls.
Guerrero-Moreno et al., 2021	Two-stage retrospective nested case-control study	To compare corneal nerve structural abnormalities on IVCM in patients with neuropathic corneal pain due to autoimmune dry eye or primary meibomian gland dysfunction.	For neuropathic corneal pain: Neuropathic corneal pain patients had greater number and larger microneuromas than healthy controls. Compared to healthy controls, patients with autoimmune dry eye disease-related neuropathic corneal pain had an increased number of microneuromas, where those with meibomian gland dysfunction-related neuropathic corneal pain had an increased number, perimeter, and area of neuromas. For dry eye disease: In patients with pain, those with concomitant autoimmune dry eye had a significantly lower number of microneuromas than those with Meibomian gland dysfunction. In patients without pain, had significantly smaller microneuroma areas and perimeter than those with meibomian gland dysfunction.
Aggarwal et al., 2019	Retrospective case-control study	To evaluate the efficacy of autologous serum tears in the treatment of neuropathic corneal pain.	Autologous serum tears had positive effects on the amelioration of corneal microneuromas. Pre-treatment, patients had a significantly higher presence of neuromas (100% of patients) than control group (no patients had microneuromas). Post-treatment, microneuromas significantly decreased and were found in 6.25% of patients.
Moein et al., 2020	Retrospective case-control study	To find an objective diagnostic sign to identify patients with neuropathic corneal pain using IVCM.	All patients with neuropathic corneal pain had microneuromas identified by IVCM, but none in dry eye disease patients or control subjects. Microneuromas detected by IVCM may serve as a sensitive and specific sign and diagnostic biomarker for neuropathic corneal pain.
D'Souza et al., 2022	Cross-sectional study	To understand the basis of discordance between symptoms of discomfort and clinical signs of ocular surface diseases.	Among patients with neuropathic corneal pain patients, those with a greater severity of ocular surface pain and discomfort symptoms had significantly more microneuroma-like structures observed on IVCM. The discordance between ocular surface discomfort and clinical signs is correlated with microneuroma-like structures detected by IVCM.
Dry eye disease			
Dermer et al., 2022	Retrospective study	To investigate the relationship between the frequency of microneuromas and clinical features of dry eye	Microneuroma frequencies did not significantly differ between subjects with and without dry eye symptoms. The presence of microneuromas alone could not distinguish the subtypes of dry eye disease. The frequency of microneuromas also could not differentiate dry eye disease resulting from refractive surgery or not.
Ren et al., 2022	Randomized double-blinded controlled trial	To demonstrate the changes in corneal nerve parameters, symptoms, and signs in dry eye disease patients after oral vitamin B1 and mecobalamin treatment.	The treatment group had significant improvements in the reduction of neuromas. Oral vitamin B1 and mecobalamin have a positive effect in ameliorating corneal nerve length, width, and neuromas in dry eye disease patients, as well as improving some symptoms and signs.
Neurotrophic keratopathy			
Yavuz Saricay et al., 2021	Retrospective case series	To illustrate that ocular pain may occur in patients with neurotrophic keratopathy.	Six out of seven stages 1 neurotrophic keratopathy patients in the study had microneuromas, alongside severely decreased corneal nerves and severe ocular pain.
Sjögren's syndrome			
Luzu et al., 2022	Retrospective case-control study	To study changes in the subbasal nerve plexus by IVCM in Sjögren's syndrome with and without small fiber neuropathy.	More subjects with Sjögren's syndrome had subbasal plexus neuromas than healthy control group subjects. The average number of neuromas per patient was significantly higher in patients (5.0 ± 7.3) compared to the control group (1.5 ± 3.1) ($P = 0.001$). More patients with Sjögren's syndrome with small fiber neuropathy had neuromas (95%) than those without.
Post-refractive surgery			
Moshirfar et al., 2021	Retrospective case series	To present a 26-year-old case series on patients with neuropathic corneal pain post-laser in situ keratomileusis.	One case was a 36-year-old Caucasian woman who underwent an uncomplicated laser-assisted in situ keratomileusis procedure, she presented to the clinic nine months post-operatively with stabbing ocular pain and IVCM examination revealed the presence of microneuromas.
Liu et al., 2020	Randomized controlled trial	To compare long-term corneal nerve status after small incision lenticule extraction versus laser in situ keratomileusis.	Corneal neuromas were seen following refractive surgery, regardless of surgical type (small incision lenticule extraction versus laser in situ keratomileusis), even five years post-operatively.
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection			
Woltsche et al., 2022	Case report	To increase awareness that SARS-CoV-2-induced neuropathic pain can occur in the cornea.	Microneuromas were found in a patient with neuropathic corneal pain secondary to SARS-CoV-2 infection seven months prior.
Barros et al., 2022	Observational retrospective study	To describe the association between SARS-CoV-2 and corneal small fiber neuropathy identified by IVCM.	There was a higher incidence of neuromas in SARS-CoV-2 patients than in healthy subjects in all age groups. The neuromas were also identified as early as three months post-polymerase chain reaction positive. The proportion of neuromas seemed to increase in patients who are diagnosed for a longer time (3–6 months and above 6 months).

Clinical Association of Corneal Neuromas with Ocular Surface Diseases

The term "neuroma" was first used to describe a feature of corneal nerves in IVCM images in 2015 in patients with photoallodynia, defined as severe ocular discomfort and sensitivity in response to regular light (Aggarwal et al., 2015). Since then, corneal neuromas have also been found to be associated with several other ocular surface diseases, including diabetic corneal neuropathy, neuropathic corneal pain, dry eye disease, neurotrophic keratopathy, Sjögren's syndrome, bullous keratopathy, as well as laser refractive surgical procedures.

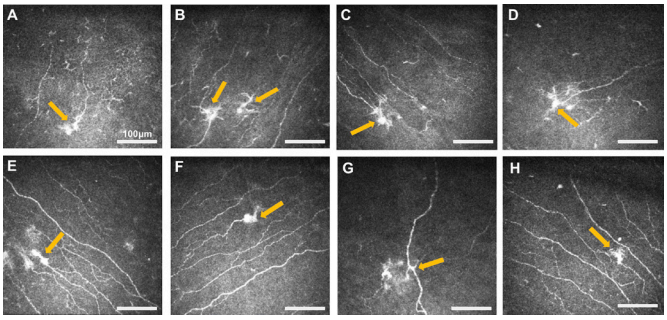

Figure 2 | **Representative *in vivo* corneal microscopy micrographs of the subbasal nerve plexus showing microneuromas.**
Images were produced via the Heidelberg retina tomograph Corneal Module (Heidelberg Engineering, Heidelberg, Germany), laser scanning confocal microscopy. Scale bars: 100 μm. (A, B) Microneuromas (arrows) are manifested as irregularly shaped, enlargements of terminal nerve endings with poorly defined margins and variable hyper-reflectivity. (C, D) Corneal nerves that terminate abruptly in a "stump microneuroma" (arrows), which are characterized as abrupt and swollen terminations of the stromal nerves (Ross et al., 2020). (E, F) Spindle microneuroma (arrows) which are described as fusiform enlargements of a corneal nerve, in the absence of axonal sprouting (Ross et al., 2020). (G, H) Lateral microneuromas (arrows), that are characterized by localized hyperreflective enlargements of a corneal nerve from which single or multiple tortuous nerves sprout (Ross et al., 2020). Unpublished data.

Association between Corneal Neuromas and Diabetic Corneal Neuropathy

Studies have found an association of type 1 and 2 diabetes mellitus (DM) with increased dendritic cell density and altered corneal nerve morphology. The dendritic cells may be a signal of inflammation since a greater number is seen in diabetic patients with coexisting chronic inflammatory demyelinating polyradiculoneuropathy (Fleischer et al., 2021). Hyperglycemia leads to infiltration by corneal dendritic cells (Liu et al., 2023), and the neuroinflammatory status in DM as well as the direct contact between dendritic cells and subbasal corneal nerves may exacerbate nerve damage and hence the manifestation of diabetic corneal neuropathy (Leppin et al., 2014). In addition, decreased corneal nerve fiber density, branch density, nerve fiber length, and nerve beading, and increased nerve tortuosity were observed in type 1 and 2 DM patients compared with control (**Figures 3A** and **B**; So et al., 2022; Teo et al., 2022b). Stump and spindle microneuromas in a patient with type 2 DM were also captured in our unpublished study (**Figure 3C**). The reduction in subbasal nerve plexus density and small-fiber neuropathic changes detected by IVCM in type 1 and 2 DM patients preceded electrophysiology or clinical symptoms of diabetic peripheral neuropathy, and studies have concluded that decreased corneal nerve fiber length was a reproducible biomarker of early diabetic peripheral neuropathy (Hertz et al., 2011). In summary, diabetic corneal neuropathy develops in early DM and the onset of corneal nerve changes precedes the progression of diabetic peripheral neuropathy. Assessing changes in corneal nerve morphology could be used to timely detect and monitor the progression of, as well as determine interventional efficacy in the management of diabetic neuropathy (Zhou et al., 2022).

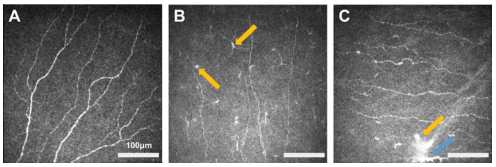

Figure 3 | **Representative *in vivo* corneal microscopy micrographs of the subbasal nerve plexus in a normal subject and a patient with type 2 diabetes mellitus (DM).**
Images were produced via the Heidelberg retina tomograph Corneal Module (Heidelberg Engineering, Heidelberg, Germany), laser scanning confocal microscopy. Scale bars: 100 μm. (A) Normal subbasal corneal nerve morphology in a subject without DM, where the hyper-reflective, continuous, non-tortuous white lines correspond to normal corneal nerves. Abnormal corneal subbasal nerve morphologies on IVCM include nerves which are apparently discontinuous, increased nerve tortuosity, reduced density of subbasal nerves, and the presence of microneuromas, keratocyte nuclei and inflammatory cells such as dendritic cells. (B) Decreased nerve density with the presence of dendritic cells (arrows) in a patient with type 2 DM. Dendritic cells were identified on IVCM evaluation by their typical presentation as the bright corpuscular particles and a diameter of up to 15 μm (Zhivov et al., 2005). The dendritic cells captured in Figure 3B likely represent immature dendritic cells, which are depicted as small, reflective cell bodies without discernible dendrites, or dendrites with an end-to-end length of shorter than 25μm. On the other hand, the morphology of a mature dendritic cell is a bright, reflective slender main cell body with many long arm-like extensions from it, and an end-to-end length of 25–45 μm or longer (Lagali et al., 2018; Liu et al., 2023). (C) Stump (yellow arrow) and spindle (blue arrow) microneuromas in a patient with type 2 DM. The stump microneuroma is identified by an abrupt and swollen termination of the stromal nerve, while the spindle microneuroma is shown by an irregularly-shaped hyper-reflective enlargement at the nerve ending. Unpublished data.

Furthermore, a clinical trial conducted by Issar et al. (2020) found increased corneal microneuromas to be correlated with poorer glucose control, suggested by the increase in percentage time in hyperglycemia and continuous overlapping net glycemic action. Continuous overlapping net glycemic action assesses intraday variability of glucose levels, and is defined as the standard deviation of the sum of differences between the current glucose recording and a recording one hour prior, with a higher continuous overlapping net glycemic action, indicating greater glycemic variation. Conversely, fewer microneuromas were observed with a greater percentage time in normoglycemia or target range. This finding may be explained by how chronic hyperglycemia causes a reduction in microvascular supply to neurons (Zhou et al., 2022) and nerve velocity (Bodman and Varacallo, 2022), leading to neural damage and denervation. The small corneal nerves lack protection from myelin, thus making them susceptible to nerve injury (Feldman et al., 2017). Corneal neuromas thus can be a potential biomarker for glucose control.

Association between Corneal Neuromas and Neuropathic Corneal Pain

Neuropathic corneal pain is due to the dysfunction of the somatosensory nervous system (Jensen et al., 2011), and can present with a complete absence of clinical ocular surface abnormalities (Galor et al., 2013; Stapleton et al., 2017). Neuropathic corneal pain results from various causes, ranging from systemic diseases (such as diabetes, small fiber neuropathy, degenerative or ischemic diseases, systemic autoimmune disease, chronic inflammatory conditions) or ocular causes (such as ocular surgeries, chronic contact lens wear, herpes simplex keratitis, radiation keratopathy, and dry eye disease) (Goyal and Hamrah, 2016). Patients typically present with lower nerve density, higher corneal nerve tortuosity and a greater number of microneuromas. The presence of microneuromas in such patients may be due to abnormal nerve regeneration after nerve injury and Wallerian degeneration of the distal segment. Wallerian degeneration refers to an active process of retrograde degeneration of an end of an axon distal to the site of the lesion (Goyal and Hamrah, 2016; Dieckmann et al., 2017). These morphological nerve changes result in a series of molecular changes, such as modified gene and protein expression, which alter the overall neuronal excitability (Belmonte et al., 2004a), which may lead to hyperalgesia, allodynia, and the discomfort symptoms of neuropathic corneal pain.

A study investigated patients who had persistent severe ocular pain for at least one year, but with an unremarkable ocular surface on clinical examination, termed "pain without stain" (Ross et al., 2020). IVCM revealed microneuromas for all patients, with an average of 4.59 microneuromas per eye. There was a significantly increased number of microneuromas in patients who responded to topical anesthesia (i.e., peripheral neuropathic corneal pain), compared with those who did not respond (i.e., central neuropathic corneal pain) ($P < 0.0001$). Spindle, lateral, and stump microneuromas were found in 74%, 44.4%, and 14.8% of eyes respectively. Representative IVCM images of neuropathic corneal pain are shown in **Figure 4**.

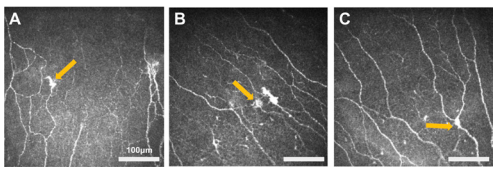

Figure 4 | ***In vivo* corneal microscopy micrographs of the subbasal nerve plexus showing corneal nerve abnormalities in patients with neuropathic corneal pain.**
Images were produced via the Heidelberg retina tomograph Corneal Module (Heidelberg Engineering, Heidelberg, Germany), laser scanning confocal microscopy. (A) Corneal nerves that terminate abruptly in a "stump microneuroma" (arrow). Scale bars: 100 μm. B: Lateral microneuroma (arrow) that are characterized by localized hyperreflective enlargements of a corneal nerve from which single or multiple tortuous nerves sprout. (C) Decreased nerve density, increased nerve tortuosity, and a spindle microneuroma (arrow), which appear as hyperreflective fusiform enlargements of the corneal nerves. Explained by a chain of molecular processes and the alteration of neuronal excitability (Belmonte et al., 2004a), these morphological corneal nerve changes can contribute to the development of symptoms in neuropathic corneal pain (Belmonte et al., 2004b; Giannaccare et al., 2017). Unpublished data.

Another study investigating neuropathic corneal pain secondary to primary meibomian gland dysfunction or autoimmune dry eye found that compared with healthy controls, patients with autoimmune dry eye disease-related neuropathic corneal pain showed an increased number of microneuromas, whereas patients with meibomian gland dysfunction-related neuropathic corneal pain had increased number, (expressed as the perimeter of each microneuroma in μm), and area perimeter (expressed as the area of each microneuroma in μm^2) of microneuromas. Microneuromas were also observed in healthy controls, but fewer and smaller than in neuropathic corneal pain patients (Guerrero-Moreno et al., 2021). These findings suggest the diagnosis of neuropathic corneal pain will require parameters beyond the number of microneuromas, such as the perimeter and area of the neuromas, which were both significantly higher in patients than in healthy controls.

An investigation of the clinical efficacy of autologous serum tears in the management of neuropathic corneal pain revealed that autologous serum tears had positive effects on the amelioration of corneal microneuromas (Aggarwal et al., 2019). Prior to treatment, the patients' study group showed significantly a higher presence of microneuromas (100% of patients) while none of the controls had microneuromas. After treatment, the presence of microneuromas were significantly decreased and were seen in 6.25% of patients. This is in line with our unpublished study where decreased microneuroma areas were observed after topical umbilical cord plasma treatment (**Figure 5**). These findings demonstrate that patients' corneal nerve abnormalities, as assessed by microneuromas in IVCM, can be improved following treatment with blood-derived products. The efficacy of autologous serum tears or plasma could be explained by the eyedrops having many neurotrophic and epithelial growth factors like nerve growth factor, which has been shown to play a vital role in the suppression of neuroinflammation and promotion of nerve health (Bradley et al., 2008; Pan et al., 2017).

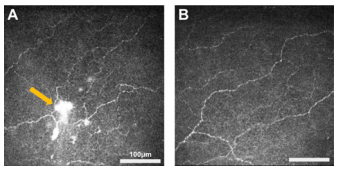

Figure 5 | **Representative *in vivo* corneal microscopy micrographs showing the corneal subbasal nerve plexus of a patient with neuropathic corneal pain before and after plasma treatment.**
Images were produced via the Heidelberg retina tomograph Corneal Module (Heidelberg Engineering, Heidelberg, Germany), laser scanning confocal microscopy. Scale bars: 100 µm. (A) Neuromas (arrow) were observed before plasma treatment (indicated by yellow arrow), shown as hyperreflective, irregular-shaped enlargements of the terminal end of a nerve. (B) A marked decrease in neuroma area was observed 6 weeks after umbilical cord plasma eyedrop treatment. This is aligned with previous literature reporting that plasma rich in growth factors (PRGF) suppresses neuroinflammation in the eye (Sanchez-Avila et al., 2018; Soifer et al., 2022). Unpublished data.

Another retrospective case-control study found that microneuromas identified by IVCM, were present in all patients with neuropathic corneal pain but none in patients with dry eye disease or controls, and therefore showing that the sensitivity and specificity of microneuromas for neuropathic corneal pain was 100% (Moein et al., 2020). The results suggest that microneuromas could serve as a highly sensitive and specific biomarker for the diagnosis of neuropathic corneal pain, in the presence of neuropathic symptoms. In addition, microneuromas were not observed in dry eye disease patients without pain, or in healthy controls. This could explain how microneuromas were responsible for ocular discomfort in neuropathic corneal pain. Morphologic nerve changes are associated with molecular changes such as modified genes and expression of proteins, which subsequently increased neuronal excitability, causing hyperalgesia, allodynia, and symptoms of neuropathic corneal pain (Belmonte et al., 2004a).

Furthermore, another study found that among neuropathic corneal pain patients, there were significantly more microneuroma-like structures observed on IVCM for those with greater severity of ocular surface pain and discomfort symptoms (D'Souza et al., 2022). In that study, a low proportion- 6 out of 50 eyes- of healthy asymptomatic subjects had microneuroma-like structures in the subbasal nerve plexus, similar to previous literature (Guerrero-Moreno et al., 2021), although a separate study reported that healthy controls did not have microneuromas (Ross et al., 2020).

Association between Corneal Neuromas and Dry Eye Disease

Dry eye disease is a common ocular surface disease with classical symptoms of visual disturbances, ocular discomfort, and pain. It is a multifactorial disease characterized by the loss of tear-film homeostasis and ocular symptoms, in which factors that contribute to it are ocular surface inflammation and damage, tear-film instability and hyperosmolarity, and neurosensory abnormalities (Craig et al., 2017). The pathogenesis of this condition is that reduced tear secretion results in inflammation and damage of peripheral nerves, triggering sensitization and abnormal corneal nerve activity, thus evoking pain, dryness, itching, and foreign body sensation (Belmonte et al., 2017). Most commonly, dry eye disease is caused by autoimmune disorders and dysfunction of the meibomian gland (Bron et al., 2017). Reported data are scarce regarding the association between corneal nerve characteristics and microneuromas with dry eye disease, as well as using corneal nerve morphological characteristics to distinguish etiological subgroups of the disease.

A recent study demonstrated that the presence of microneuromas could not distinguish the subtypes of dry eye disease, and the frequency of microneuromas could not differentiate the dry eye disease resulting from refractive surgery or not (Dermer et al., 2022). However, another study conducted (Guerrero-Moreno et al., 2021) evaluated the corneal nerve abnormalities in patients with autoimmune dry eye or primary meibomian gland dysfunction, which are the most common etiologies of dry eye disease (Bron et al., 2017). The patients were further divided by whether they were painless or had associated neuropathic corneal pain symptoms. In the patient cohorts with pain, those with concomitant autoimmune dry eye had a significantly lower number of microneuromas than those with meibomian gland dysfunction. Furthermore, in the patient groups without pain, those with autoimmune dry eye had significantly smaller microneuroma areas and perimeter than those with meibomian gland dysfunction. This implies that more detailed analyses, such as the quantification of the area and perimeter of microneuromas, are required to better differentiate corneal nerve changes in these groups of dry eye disease patients.

In a randomized controlled trial which investigated the therapeutic potential of oral vitamin B1 and mecobalamin in the treatment of dry eye disease, the number of corneal neuromas were significantly lower ($P < 0.05$) in the treatment group than the controls, after 1 and 3 months of treatment, alongside improvement in dryness and other classical signs and symptoms of dry eye disease (Ren et al., 2022). This could be attributed to how vitamin B12, which mecobalamin is a coenzyme form of, promotes β-III tubulin expression in neurons and is involved in the regulation of neurotrophic factor synthesis, contributing to neurite outgrowth and promoting epithelial wound healing. Additionally, vitamin B1 improves axonal flow and transport indirectly and helps damaged nerve tissue to be repaired.

Association between Corneal Neuromas and Neurotrophic Keratopathy

Neurotrophic keratopathy is a corneal neurodegenerative disease as a result of trigeminal nerve damage, resulting in corneal sensory loss, decreased tear production and blink rate, corneal epithelial keratopathy or defect, ulceration, and eventually perforation in severe cases (Lambiase and Sacchetti, 2014). It is commonly caused by chronic use of eye drops containing preservatives, post-herpetic infections, contact lens wear, burns, and post-corneal or ocular surgery. Other causes include DM, multiple sclerosis, as well as refractive surgery (Mansoor et al., 2020). A retrospective case series (Yavuz Saricay et al., 2021) presented that six out of seven stage 1 neurotrophic keratopathy patients in the study, had the presence of microneuromas alongside severely decreased corneal nerves and clinical symptoms of severe ocular pain. The findings suggest that the presence of these microneuromas, defined in this study as morphologically abnormal corneal nerves with irregularly-shaped enlargements of subbasal nerve endings, may serve as an objective biomarker to diagnose neurotrophic keratopathy. A feature is severe epithelial damage, which aligns with the pathophysiology of microneuromas as described above (Dieckmann et al., 2017).

Association between Corneal Neuromas and Sjögren's syndrome

Changes in the subbasal nerve plexus by IVCM were reported (Luzu et al., 2022) in patients with Sjögren's syndrome, a chronic autoimmune systemic disease characterized by immune cells infiltrating exocrine glands (Bron et al., 2017). More subjects (77%) than healthy control group subjects (40%) had subbasal plexus neuromas. The average number of neuromas per patient was significantly higher in patients compared with the control group ($P = 0.001$). Moreover, more patients with Sjögren's syndrome with small fiber neuropathy had neuromas (95%) than those without it. There was also a significantly greater average number of neuromas in Sjögren's syndrome patients with associated small fiber neuropathy than patients without small fiber neuropathy ($P = 0.008$). This demonstrates that IVCM was useful to detect corneal nerve changes in Sjögren's syndrome patients, and together with other parameters such as the density of inflammatory cells, density of subbasal nerves, and neuroma identification, can potentially enable earlier diagnosis of this condition.

Corneal neuromas were described in 40% of patients with dry eye secondary to Sjögren's syndrome (Tuominen et al., 2003). As the inflammation in Sjögren's syndrome leads to decreased tear production, it progressively affects all components of the ocular surface, including the subbasal nerve plexus (Rolando, 2001). The high prevalence of neuromas in the subbasal plexus in patients with both Sjögren's syndrome and small fiber neuropathy could be the result of the small nerve fibers being targeted, and the increased number of neuromas in the subbasal plexus in Sjögren's syndrome reflects attempts at axonal regeneration (Albers et al., 1994; Streppel et al., 2002; Tuominen et al., 2003). Hence, the presence of neuromas may pose as a diagnostic marker, allowing early identification and thus the treatment and prevention of ocular and systemic complications of Sjögren's syndrome.

Association between Corneal Neuromas and Post-Refractive Surgery

Laser refractive surgery, where refractive errors are corrected by surgically removing corneal stromal tissue to change the shape and refractive power of the cornea, is one of the most commonly performed ophthalmic procedures globally (Bandeira et al., 2019). Following laser refractive surgery, neuromediators and cytokines are released by corneal nerves, epithelial and stromal cells. These soluble factors are vital for corneal nerve regeneration and wound healing after refractive surgery (Chin et al., 2021; Yang et al., 2021). As a result of the denervation and nerve regeneration processes post-surgery, refractive surgery has been shown to have an impact on corneal

nerve morphology such as the presence of neuromas, increased tortuosity of fibers, and lower nerve density compared to controls, and IVCM is a useful imaging technique to capture post-operative inflammation and monitoring of complications (Stewart et al., 2021). In particular, a reported case was a 36-year-old Caucasian woman who underwent an uncomplicated laser-assisted *in situ* keratomileusis procedure and presented to the clinic nine months post-surgery with stabbing ocular pain. IVCM examination revealed the presence of microneuromas (Moshirfar et al., 2021).

In a cross-sectional study published by our group, the presence of corneal nerve fiber alterations such as decreased corneal nerve density was observed five and a half years after surgery compared to control group subjects, suggesting that the nerve status had not recovered to normal ranges post-operatively (Liu et al., 2020). From that study, IVCM images also revealed the presence of neuromas in all the eyes even five years following refractive surgery, regardless of the type of the refractive surgery, such as laser in situ keratomileusis and small incision lenticulate extraction. **Figure 6** shows neuromas seen on IVCM following three different types of refractive surgeries – laser in situ keratomileusis, small incision lenticulate extraction, and laser-assisted subepithelial keratectomy.

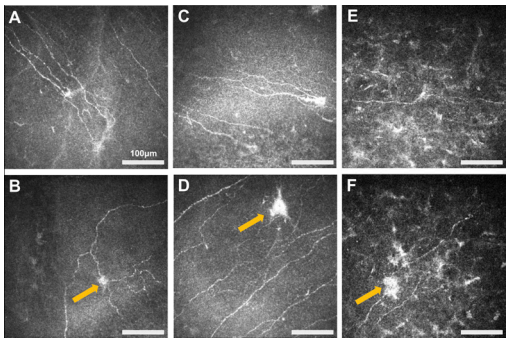

Figure 6 | **Representative *in vivo* corneal microscopy micrographs showing the corneal subbasal nerve plexus and neuromas in patients following refractive surgery.** Images were produced via the Heidelberg retina tomograph Corneal Module (Heidelberg Engineering, Heidelberg, Germany), laser scanning confocal microscopy. Scale bars: 100 µm. (A, B) Significantly decreased density and increased tortuosity of nerves with the presence of neuroma (arrow) 2 months after laser in situ keratomileusis. (C, D) Slight reduction in corneal nerves with neuroma (arrow) 2 months after small incision lenticulate extraction. (E, F) Presence of hyperreflectivity in the anterior corneal stroma with decreased nerve density and neuroma (arrow) 2 months after laser-assisted subepithelial keratectomy. Unpublished data.

Corneal Neuromas in Other Ocular Surface Diseases

IVCM can also be used to study severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced ocular surface complications. A recent publication (Barros et al., 2022) showed a higher incidence of neuromas in SARS-CoV-2 patients (65.2% of patients) than in healthy subjects across all age groups. Neuromas were identified as early as three months post-polymerase chain reaction positive. The majority of patients who had neuromas were in the 36–55-year-old age group, and the proportion of neuromas were also higher in patients diagnosed longer ago (3–6 months and > 6 months). These findings suggest that SARS-CoV-2 infection correlates with the degeneration of corneal nerve endings and small fiber neuropathy. This could be explained by the presence of neuropilin receptors 1 and 2 present in corneal nerves endings, which are the receptors for SARS-CoV-2 and may act as a gateway for the virus to infect (Liu et al., 2020a; McFarland et al., 2021). This virus also has low or absent angiotensin-converting enzyme 2 expression, resulting in the loss of the neuroprotective role of angiotensin-converting enzyme 2, reducing the ability of the axonal endings of the sensory neurons of the cornea to survive and regenerate (Bennion et al., 2015; Dolatshahi et al., 2021).

A case report described a patient presenting with ocular pain seven months following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (Woltsche et al., 2022). On IVCM examination, there were corneal microneuromas, identified by their hyperreflectivity and irregular swelling of subbasal nerve endings in both eyes (Woltsche et al., 2022). Thus, post-SARS-CoV-2 corneal nerve fiber alterations presenting as microneuromas coupled with severe ocular discomfort lead to a diagnosis of SARS-CoV-2-induced neuropathic corneal pain.

Future Perspectives

While the discovery and evaluation of corneal neuromas in ocular diseases have gained much traction and discussion in recent years, more work could be done to further understand the underlying molecular mechanisms of neuroma formation as such data remains scarce. Microneuromas release inflammatory mediators and cytokines that modify the transduction of ion channels and upregulate nociceptor ion channels, modification of membrane potentials and aberrant nerve activity, which renders nociceptors hypersensitive to stimuli, leading to peripheral sensitization (Aggarwal et al., 2019). While this explains how symptoms of pain and dysesthesia can present in patients with ocular conditions which involve corneal nerve injury and concurrently present with microneuromas, it would be helpful to further investigate the molecular evidence on neuromas, which can be the targets of therapeutic drug development.

To further elucidate the pathophysiologic mechanisms of neuromas, utilizing animal models should be considered since experimentation on human subjects' corneas in clinical settings is limited. Harnessing a suitable animal model for investigations would not only help improve knowledge of the underlying processes of corneal neuroma formation, but also provide a platform to test novel therapeutic modalities. Animal studies have demonstrated that neuromas are common after nerve injury, alter nerve excitability and are sources of ectopic impulse generation (Matzner and Devor, 1994). Murine models have been used commonly in studies of neuropathic corneal pain, and one such model showed microneuromas in the central cornea (Xiang et al., 2017). However, such a method would involve damaging the ocular surface and heavily inducing corneal inflammation, which is likely to result in immunological and wound healing processes which would interfere with the investigation of underlying mechanisms. Future direction would hence include reducing dependence on animal models and using *in vitro* three-dimensional, human-based tissue models. Examples of which include chick embryo corneas in a co-culture system with embryo trigeminal ganglia (Kubilus and Linsenmayer, 2010). This may produce higher accuracy of results by decreasing animal behavior variances and inter-species physiological variability, exemplified by the difference in corneal thickness such as the bovine and porcine corneas being around twice as thick as the human cornea.

In terms of the treatment of neuromas, a wider variety of topical agents could be investigated for their efficacies in the formation and density of corneal neuromas. Despite a study showing the therapeutic benefit of autologous serum tears and our unpublished study on umbilical cord plasma, common ocular surface treatments including artificial tears, steroids, and cyclosporine could be considered for further testing, given their proven clinical safety and are already being used widely. Examples of drugs include lifitegrast- a lymphocyte function-associated antigen-1 antagonist- in the context of dry eye disease, and cenegermin- a recombinant form of human nerve growth factor- in neurotrophic keratitis.

Regarding the IVCM imaging technique, the current limitation is IVCM does not provide a tracking feature to locate and re-scan the exact same location. We used an established method to overcome such a limitation, by selecting five non-overlapping areas (namely, center and four quadrants) to allow for the best representation of the cornea (Chin et al., 2020, 2021; Liu et al., 2020b; Teo et al., 2022b). Wide-field or large-area scanning of the corneal nerve plexus has also been proposed to address this limitation (Edwards et al., 2012).

Currently, the studies discussed in this article involve the manual identification of neuromas. It is widely accepted that the quantification of nerve parameters (Zhang et al., 2022), including the detection of neuromas (Wu et al., 2019) is consistent. However, manual methods like ImageJ require users to manually identify and delineate the neuromas before quantification. It is therefore user-dependent, labor-intensive, highly subjective, and prone to intra- and inter-observer inconsistencies especially when images have poor contrast or noisy background, where neuromas are not easily discernible and difficult to apply in a clinical setting where large amounts of data need to be analyzed (Chin et al., 2020). With growing interest in identifying and quantifying neuromas, continual improvement of imaging and quantification technologies would enable more efficient and accurate analysis of these structures. For example, an artificial intelligence model (Kundu et al., 2022) was used to analyze various corneal nerve parameters using IVCM in patients presenting with ocular surface pain for classification, where microneuromas were detected as the parameter with the highest importance by the model. The results showed that the microneuromas were an important feature to classify subjects with ocular discomfort, greater than clinical signs, hence raising the potential for artificial intelligence to be used. Hence, the development of automated software to evaluate neuromas, alongside other corneal nerve parameters and symptomatology, in a wider range of ocular surface diseases should be considered. In this way, a larger volume of IVCM images can be evaluated, paving the way for a deeper understanding of symptoms and thus effective treatment plans for patients (Recchioni et al., 2020). Additionally, more work could be done to provide further quantification (such as area, perimeter, and length) or phenotypic description of neuromas, because a majority of current literature reports the presence or absence of neuromas as a biomarker for ocular diseases, which is non-specific as this review has shown that it could be seen in a wide variety of conditions.

Conclusion

This article has reviewed current studies which report the association between neuromas and various ocular surface diseases. Corneal neuromas are formed from pathological or mechanical damage to nerve fibers, and can result in the presentation of neuropathic symptoms or sensory dysfunction in patients. Many studies have validated the potential use of corneal neuromas identified on IVCM as a surrogate marker for ocular surface diseases and for diagnosis or disease stratification. Future studies may extend to exploring the molecular basis of neuroma formation and progression, as well as using artificial intelligence software to evaluate corneal neuromas, and apply it to the monitoring and treatment efficacy of ocular surface diseases.

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