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Autologous Serum Tears for Treatment of Photoallodynia in Patients with Corneal Neuropathy: Efficacy and Evaluation with In Vivo Confocal Microscopy

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Abstract

Objective—Patients suffering from corneal neuropathy may present with photoallodynia; i.e., increased light sensitivity, frequently with a normal slit-lamp examination. This study aimed to evaluate the efficacy of autologous serum tears (AST) for treatment of severe photoallodynia in corneal neuropathy and correlate with corneal subbasal nerve alterations by in vivo confocal microscopy (IVCM).

Methods—Retrospective case control study with 16 patients with neuropathy-induced severe photoallodynia compared to 16 normal controls. Symptom severity, clinical examination and bilateral corneal IVCM scans were recorded.

Results—All patients suffered from extreme photoallodynia (8.8 ± 1.1) with no concurrent ocular surface disease. Subbasal nerves were significantly decreased at baseline in patients compared to controls; total nerve length (9208 ± 1264 vs 24714 ± 1056 $\mu\text{m}/\text{mm}^2$; $p < .0001$) and total nerve number (9.6 ± 1.4 vs 28.6 ± 2.0 ; $p < .0001$), respectively. Morphologically, significantly increased reflectivity (2.9 ± 0.2 vs 1.8 ± 0.1 ; $p < .0001$), beading (in 93.7%), and neuromas (in 62.5%) were seen. AST (3.6 ± 2.1 months) resulted in significantly decreased symptom severity (1.6 ± 1.7 ; $p = .02$). IVCM demonstrated significantly improved nerve parameters ($p < .005$), total nerve length

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($15451 \pm 1595 \mu\text{m}/\text{mm}^2$), number (13.9 ± 2.1), and reflectivity (1.9 ± 0.1). Beading and neuromas were seen in only 56.2% and 7.6% of patients.

Conclusion—Patients with corneal neuropathy-induced photoallodynia show profound alterations in corneal nerves. AST restores nerve topography through nerve regeneration, and this correlated with improvement in patient-reported photoallodynia. The data support the notion that corneal nerve damage results in alterations in afferent trigeminal pathways to produce photoallodynia.

Keywords

autologous serum tears; corneal neuropathy; laser in vivo confocal microscopy; light sensitivity; nerve growth factor; photoallodynia; regeneration

I. INTRODUCTION

Corneal neuropathy, a recently described entity, is characterized by dysfunctional nerves following direct damage to the trigeminal nerve endings.¹ Chronic and persistent ectopic activity of injured corneal nerves causing pain in response to both innocuous stimulation (allodynia) and noxious stimuli (hyperalgesia) of the cornea is the hallmark feature of neuropathy, with patients typically reporting severe spontaneous corneal pain.^{1–3} In addition, a large number of these patients may experience increased light sensitivity, which may serve as a proxy symptom to the pain. The severe ocular discomfort in response to light in these neuropathic patients is referred to as *photoallodynia*, which has a serious debilitating impact on the quality of life of the patients.^{2,3} Patients may be unable to carry out activities of daily living, even when wearing dark glasses, resulting in loss of work hours, impaired social functioning, frustration, and increased anxiety and depression.⁴

Despite the severe impact on the quality of life, corneal neuropathy-induced photoallodynia is often overlooked by ophthalmologists and remains severely undertreated.⁴ Because evidence of nerve injury is required for the diagnosis of neuropathy, routine slit-lamp biomicroscopy does not allow for diagnosis of this condition. The recent advent of laser in vivo confocal microscopy (IVCM), which is a noninvasive, high-resolution, real-time imaging device, has allowed visualization of the cornea at 800-times magnification with a lateral digital resolution of $1 \mu\text{m}/\text{pixel}$. Layer-by-layer analysis of the corneal ultrastructure in both ocular health and disease, including detailed visualization of corneal nerves and immune cells, is now possible. Both quantitative and qualitative assessments of the cellular and nerve properties can be performed and provide insight into the underlying disease pathogenesis, diagnosis, and efficacy of treatments.^{5–14} Very recently, laser IVCM has demonstrated corneal nerve abnormalities in patients with severe symptoms of pain or photoallodynia, despite the lack of clinical signs on slit-lamp examination, thus prompting a diagnosis of corneal neuropathy.^{2,3,15–17}

Given that the basis of the extreme corneal neuropathy-induced photoallodynia is associated with nerve injury, therapeutic strategies resulting in regeneration of damaged nerves may help alleviate patient symptoms. This rationale is based on reports on the use of neurotrophic factors, particularly nerve growth factor (NGF) to reduce allodynia in animal models with

neuropathic pain.^{18,19} By reducing reactive astrocytosis and reversing the glial morphomolecular modulation, NGF reduces both allodynia and hyperalgesia. Administration of neurotrophic factors results in post-injury repair of peripheral nerves and their functional recovery.²⁰ Successful corneal nerve regeneration following the use of autologous plasma in neurotrophic keratopathy,²¹ as well as with autologous serum tears (AST) in dry eye patients, prompted us to consider the possible application of AST in patients suffering from corneal neuropathy-induced photoallodynia.²²

We hypothesized that AST would result in corneal nerve regeneration and subsequent functional recovery of the damaged peripheral corneal nerves in patients suffering from corneal neuropathy, leading to symptomatic improvement in photoallodynia. The purpose of this pilot study is to assess corneal nerve changes in patients with corneal neuropathy associated with photoallodynia and to evaluate the clinical efficacy of AST therapy as correlated with IVCM.

II. METHODS

A. Study Design and Patients

All subjects were recruited from the Cornea Service of the Massachusetts Eye & Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, between 2009 to 2012. The protocol was approved by the Institutional Review Board/ Ethics Committee, complied with the Health Insurance Portability and Accountability Act (HIPAA), and adhered to the tenets of the Declaration of Helsinki.

This was a retrospective, single-center study. Sixteen patients (10 females and 6 males, mean age 61.8 ± 4.4 , range 27–88 years) with the diagnosis of corneal neuropathy were included in the study and were compared to 16 controls (10 females and 6 males, mean age 56.4 ± 3.1 , range 25–74 years; $p=0.6$). Inclusion criteria included symptoms of severe photoallodynia, a presence of a normal slit-lamp examination, and the absence of clinical signs of ocular surface disease by slit-lamp examination at the time of baseline examination. Specific exclusion criteria included any anterior or posterior segment pathology that could independently cause the symptoms of light sensitivity, such as corneal abrasion, ulcer, ocular infections, uveitis, and iridocyclitis. All the patients had tried frequent lubrication for at least 3 months without any relief in symptoms of photoallodynia, and were currently being treated with 20% AST 8 times/day for the purpose of inducing corneal nerve regeneration, in order to address symptoms of photoallodynia. Both eyes of all patients were studied; however, only one eye was randomly selected for analysis.

B. Clinical Chart Review

The charts of all the patients were reviewed for symptoms, activities of daily life that were affected, and previous ocular history. The severity of photoallodynia symptoms was recorded based on the patient's assessment on a scale of 0–10, 10 being the maximum. Results of ophthalmic examination, including slit-lamp biomicroscopy findings, corneal fluorescein staining, conjunctival lissamine green staining, Schirmer's test with anesthesia, and tear film breakup time (TFBUT) were recorded in addition to the treatment regimen and

duration of therapy. Two time points, before (baseline) and after treatment with 20% autologous serum drops were assessed.

C. In Vivo Confocal Microscopy

Laser IVCM (Heidelberg Retina Tomograph 3 with the Rostock Cornea Module [HRT/RCM], Heidelberg Engineering GmbH, Heidelberg, Germany) of the central cornea was performed in all patients bilaterally. Laser IVCM is performed routinely for all patients who present to our clinic with ocular surface disease, pain, or light sensitivity at baseline and follow-up visits, as an adjunct device for the assessment of corneal nerves and immune cell alterations in the cornea, given minimal slit-lamp findings in many patients. Only one randomly selected eye was chosen for quantitative analysis and was compared to the central corneas of normal age- and gender-matched reference controls as previously described.⁷

The microscope uses a 670-nm red wavelength diode laser source and is equipped with a 63× objective immersion lens with a numerical aperture of 0.9 (Olympus, Tokyo, Japan). A coronal section of the cornea of 400×400 μm (horizontal × vertical) is represented by each image. Adjacent images are separated by 1 μm, with a lateral resolution of 1 μm/pixel. Digital images are recorded at a speed of 30 frames/second. During confocal microscopy scanning, a disposable, sterile polymethylmethacrylate cap (Tomo-Cap; Heidelberg Engineering GmbH, Heidelberg, Germany), filled with a layer of hydroxypropyl methylcellulose 2.5% (GenTeal gel; Novartis Ophthalmics, East Hanover, NJ) in the bottom, is mounted on the cornea module. Both eyes are anesthetized using one drop of topical anesthesia, 0.5% proparacaine hydrochloride (Alcaine; Alcon, Fort Worth, TX), followed by a drop of hydroxypropyl methylcellulose 2.5% (GenTeal gel, Novartis Ophthalmics). One drop of hydroxypropyl methylcellulose 2.5% is also placed on the outside of the cap to improve optical coupling. The equipment is manually advanced until the gel on the cap comes in contact with the surface of the central cornea.

Six to eight scans were performed on the full thickness of the central cornea using the sequence mode. With this approach, the subbasal plexus is observed in the subepithelial area, immediately at or posterior to the basal epithelial layer and anterior to the Bowman's layer, typically at a depth of 50–80 μm. Three images most representative of the subbasal nerve plexus were chosen for analysis by a masked observer. The criteria for selecting the images were the best focused images, in a single layer, without folds, and good contrast. Two masked observers then evaluated the confocal images for morphology and density of subbasal nerves.

The nerve analysis was performed using the semiautomated tracing program NeuronJ, (<http://www.imagescience.org/meijering/software/neuronj/>)²³ a plug-in for ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/>) as previously described.⁵ Metrics were assessed as described below..

1. *Total nerve density* was assessed by measuring the total length of the nerve fibers in micrometers per frame (160,000 μm² [Figure 1]).

2. Main nerve trunks were defined as the total *number of main nerve trunks* in one image after analyzing the images anterior and posterior to the analyzed image to confirm that these did not branch from other nerves.
3. *Nerve branching* was defined as the total number of nerve branches in one image.
4. The number of *total nerves* measured was defined as the number of all nerves, including main nerve trunks and branches, in one image. The data were expressed as density ($\mu\text{m}/\text{mm}^2$) \pm SEM.
5. The grade of *nerve tortuosity* and reflectivity was classified in four grades according to a *relative grading scale* reported by Oliveira-Soto and Efron.²⁴
6. *Neuromas* represent stumps of severed nerves and were identified as abrupt endings of a nerve fiber on confocal images.
7. *Beading* was defined as discrete small beads along the length of the nerves.²⁴ The presence of beading and neuromas was reported qualitatively as present or absent.
8. The images were also analyzed for the density of the *dendritiform cells (DCs)* present in the subbasal layer using the cell counter function in Image J, as previously described.⁷

D. Preparation and Administration of Autologous Serum Tears

Patients were tested for blood-borne infections, including hepatitis B and C, syphilis, and HIV serology. A positive serology excluded the patients from use of AST. 100 ml of whole blood was drawn by venipuncture at the antecubital fossa under aseptic conditions and left standing for 2 hours to ensure complete clotting. The blood was then centrifuged for 15 minutes at 3000 rpm. The serum was separated in a sterile manner and filtered using MILLLEX-HP PES 5 micron low protein binding filter and diluted to 20% using sterile saline solution. The final preparation was allocated into 5 ml bottles. The bottles were kept frozen at -20°C until ready for use. After thawing, expiration was 2 weeks (unopened) and 1 week (after opening). To protect from ultraviolet light, an amber colored sleeve was provided with the bottles. The patients were instructed to keep the bottles under refrigeration. AST was applied 8 times daily bilaterally.

E. Statistical Analysis

Statistical analysis was performed by analysis of variance (ANOVA) to compare the pre-treatment, post-treatment, and control groups. Paired Student's *t*-test was used to compare pre- and post-treatment groups. Differences were considered statistically significant for *p* values less than 0.05. Analyses were performed with statistical analysis software in STATA™ (StataCorp, version 11).

F. Literature Review

A literature review was performed in PubMed to confirm the novelty of use of autologous serum drops for treatment of corneal neuropathic symptoms-- specifically, increased light sensitivity or photoallodynia-- and the correlation with in vivo confocal microscopy findings. The following search terms were used: corneal neuropathy, light sensitivity,

photophobia, photoallodynia, autologous serum, corneal nerve regeneration with autologous serum in neuropathy, in vivo confocal microscopy and neuropathy, in vivo confocal microscopy and nerves.

III. RESULTS

A. Demographics

The cornea of one of the eyes in each of the 16 patients suffering from severe photoallodynia were studied and the data collected was compared with 16 age-matched control eyes. Demographic data of patients and controls are presented in Table 1.

B. Clinical Features

All patients complained of increased and debilitating photoallodynia. Severity of symptoms ranged from 7 to 10, with a mean of 8.8 ± 1.1 . The impact on quality of life of patients was significant. All patients reported difficulty in reading, watching TV, and driving. Eleven patients (64.7%) were dependent on sunglasses, even indoors. Four of 16 patients (25%) lost their jobs and one had to drop out of graduate school due to inability to tolerate any form of light. Patients also reported significant social impairment, as they tended to spend days in dark rooms, refusing to tolerate any form of artificial or natural light. Severity of symptoms, activities affected, impact on quality of life, and the possible underlying mechanism leading to corneal neuropathy are shown in Table 2.

None of the patients suffered from a concurrent active ocular surface disease, as evidenced by absence of clinical signs, including fluorescein and lissamine green staining, decreased Schirmer's test results, or TFBUT. Slit-lamp biomicroscopy of the eyelid margins and conjunctiva did not reveal any active blepharitis, meibomian gland dysfunction, or conjunctivitis. Twelve eyes had a best-corrected visual acuity of 20/20, two had 20/25, while the other two eyes were 20/30 and 20/50 due to cataracts.

Following treatment with AST, the patients reported a significant reduction in symptoms, with 9/16 patients (56.25%) demonstrating more than 90 % improvement and the remaining 7/16 reporting 40–60% improvement in the symptom of photoallodynia. The average symptom severity was reduced significantly to 1.6 ± 1.7 (range 0–5; $p = .02$). Figure 2 demonstrates the improvement in symptom severity post-treatment. The mean interval for improvement was 3.6 ± 2.1 months, ranging from 1 to 8 months. The severity of symptoms post-treatment with AST, percentage improvement, and duration for improvement are presented in Figure 2 and Table 2.

C. In Vivo Confocal Microscopy Findings

A summary of the nerve parameters for the patients, pre- and post-treatment with AST, and normal control groups is presented in Table 3. Figure 3 shows the improvement in corneal subbasal nerve plexus for three representative patients following treatment.

1. Pre-Treatment Baseline IVCM Findings—The subbasal nerve plexus density in patients pre-treatment was profoundly decreased compared to controls (Figure 4), including total nerve length ($9,208 \pm 1,264 \mu\text{m}/\text{mm}^2$ vs $24,714 \pm 1,056 \mu\text{m}/\text{mm}^2$; $p < .0001$), total number

of nerves (9.6 ± 1.4 vs 28.6 ± 2.0 ; $p < .0001$), main nerve length ($3,958 \pm 460 \mu\text{m}/\text{mm}^2$ vs $11,214 \pm 602 \mu\text{m}/\text{mm}^2$; $p < .0001$), number of main nerves (1.6 ± 0.2 vs 4.3 ± 0.2 ; $p < .0001$), nerve branch length ($5,483 \pm 838 \mu\text{m}/\text{mm}^2$ vs $13,519 \pm 807 \mu\text{m}/\text{mm}^2$; $p < .0001$), and number of nerve branches (7.9 ± 1.2 vs 24.3 ± 2.0 ; $p < .0001$), respectively. Morphologically, the nerves showed significant increase in reflectivity (2.9 ± 0.2 vs 1.8 ± 0.1 ; $p < .0001$), and demonstrated the presence of beading and neuromas in 93.7% and 62.5% of patients, respectively. The nerves also showed increased tortuosity (2.2 ± 0.1), although this was not significant compared to controls (1.8 ± 0.2 ; $p = .06$). There was no significant difference in the density of DCs compared to controls (54.2 ± 21.3 vs 17.3 ± 4.4 cells/ mm^2 ; $p = .1$).

2. Post-Treatment IVCM Findings—IVCM showed a significant improvement in nerve density (total nerve length $15,451 \pm 1595 \mu\text{m}/\text{mm}^2$ and number 13.9 ± 2.1 , main nerve length $6,525 \pm 601 \mu\text{m}/\text{mm}^2$ and number 2.6 ± 0.2 , branch nerve length $8,925 \pm 1,228 \mu\text{m}/\text{mm}^2$ and number 11.3 ± 1.9 ; $p < 0.005$) after therapy with AST (Figure 3). The density of total nerves, main nerve trunks, and nerve branches increased by 68%, 65%, and 63%, respectively. In addition to the changes in density (Figures 3 and 4), IVCM demonstrated significant improvements in nerve morphology after therapy with AST (Figures 3 and 5). A significant decrease in reflectivity (1.9 ± 0.1 ; $p = .001$) and tortuosity (1.7 ± 0.2 ; $p = .001$) of nerves was noted. Beading and neuromas were now seen in only 56.2% and 7.6% of patients, respectively, a decrease of 37.5% and 56.25%, respectively ($p = .01$) compared to pre-treatment. These changes are reported at the time point when patients reported the first symptomatic improvement in photoallodynia, average being 3.8 ± 0.5 months, with a range of 1–8 months.

There was no significant difference in the DC density after treatment 29.2 ± 8.4 cells/ mm^2 ; $p = .1$). When clinical symptom severity was correlated to IVCM parameters, a significant negative correlation was found between photoallodynia symptom severity and total nerve density ($r = -0.61$, $p = .002$). Similarly, significant positive correlation was seen between symptom severity and reflectivity of nerves ($r = 0.41$, $p = .02$). However, no significant correlation was seen between symptoms and tortuosity of nerves ($r = 0.17$, $p = .35$).

IV. DISCUSSION

In the current study, all patients suffered from extreme photoallodynia. Despite their severe symptoms, the clinical signs on slit-lamp examination were unremarkable, but decreased nerve density and abnormal nerve morphology was observed as measured by IVCM. Such changes are consistent with corneal neuropathy.^{2,3,16} In addition, these patients demonstrated decreased or diminished photoallodynia with application of proparacaine, suggesting that neural blockade of afferent input from the cornea is the contributing factor to the photoallodynia.

Corneal neuropathy-induced photoallodynia should be differentiated from other etiologies, including traumatic and inflammatory ocular conditions. Both anterior segment pathology, such as corneal diseases, iritis, iridocyclitis, and blepharitis, as well as posterior segment pathology, such as uveitis, vitritis, retinitis pigmentosa, and retinal and cone dystrophies (reviewed by Digre and Brennan⁴) can result in photophobia. Various neurological

conditions can result in photophobia, including headaches (most commonly migraine headaches), benign essential blepharospasm, and trigeminal neuralgia, as well as intracranial conditions, including meningitis, subarachnoid hemorrhage, and pituitary tumors.⁴ A clear history and clinical examination can help to rule out the above conditions. Further, corneal neuropathy-induced photoallodynia does not improve with cycloplegic drops or ultraviolet filters, and use of these may aid in clinical differentiation of the underlying etiology.³

IVCM can reveal corneal subbasal nerve damage and aberrant nerve regeneration and has shown excellent repeatability in recent studies.^{25,26} Both decreased density of nerves and morphological changes have been described in various ocular surface diseases.⁵⁻¹⁴ Formation of neuromas, seen as abrupt swelling of injured nerve endings⁵ and neurite sprouting^{27,28} are seen after injury and abnormal regeneration of nerves. In addition, increased beading patterns have been hypothesized to represent the mitochondria and are increased in response to metabolic stress.²⁴ Further, increased reflectivity and tortuosity of corneal subbasal nerves are seen following chronic damage in various conditions, including dry eye disease,²⁹ diabetes, and systemic neuropathies.^{30,31} Moreover, discontinuous nerves and decreased nerve density have been shown in post-refractive surgery patients.³² Finally, several studies have demonstrated profoundly decreased density and altered morphology of nerves in patients suffering from corneal neuropathy.^{1,15,16}

In the current study, IVCM demonstrated profound damage to the corneal nerves in patients with photoallodynia. The density of total nerves, main nerves, and nerve branches were all decreased in patients compared to controls. Further, morphologically, the nerves showed increased reflectivity. In addition, corneal nerves were more tortuous, albeit the difference was not significant compared to controls; this could be due to the relatively small sample size. Finally, beading and neuromas were present in most patients. However, after application of AST for a mean duration of 3.6 months, patients demonstrated significant improvement in the severity of symptoms of photoallodynia, which resulted in improved quality of life within months. Concurrently, IVCM demonstrated significant increase in nerve density and reversal of morphological alterations noted at baseline, including decreased presence of beading and neuromas.

We demonstrated that the clinical symptom severity of photoallodynia was significantly correlated to decreased total nerve density and increased reflectivity of nerves, suggesting that providing neurotrophic support and subsequent nerve regeneration achieves alleviation of symptoms. Several experiments in rat models have shown that administration of growth factors, including NGF and NGF-mimetic factors, help to reduce symptoms of neuropathic pain.^{18,19} NGF plays a role in survival, differentiation, and growth of sensory and sympathetic neurons.^{33,34} NGF has, further, a direct effect on regeneration of neurons and restoring function in injured neuronal cells.³⁴ Matsumoto et al reported that NGF concentration in autologous serum is several times higher than that in tears and reported an improvement in corneal sensation following topical serum use after a mean duration of 15 months.³⁶

In addition to NGF, AST supplies the eye surface with several epithelial and neurotrophic growth factors, such as vitamin A, epidermal growth factor, transforming growth factor β ,

fibronectin, substance P, and insulin-like growth factor-1. More recently, Rao et al demonstrated improvement in corneal nerve density following autologous plasma use.²¹ However, although therapy with serum drops is safe and no substantial side effects have been reported, it is currently unclear whether a single factor or a combination of several factors are responsible for the therapeutic effects of AST.

There may be skepticism regarding the success of AST in the treatment of corneal neuropathy,³⁶ as it is possible that AST leads to the epithelial healing as well. A healthy epithelium could potentially also result in nerve regeneration, as shown by in vitro studies on rabbit corneas.³⁷ However, we would like to point out that all our patients had no active ocular surface disease at the time of initiation of AST therapy. While approximately half of the patients had been treated for ocular surface disease in the past, including the use of topical steroids, they continued to experience severe symptoms of photoallodynia, despite resolution of ocular surface disease; this suggests that their symptoms were likely related to chronic nerve changes as observed by IVCN. Further, the fact that the patients with no history of ocular surface disease had similar IVCN findings of nerve degeneration and demonstrated a similar response and improvement in symptoms suggests that AST may produce patient improvement through nerve regeneration.

Inflammation can produce corneal pain and symptoms of photoallodynia. At the TFOS (Tear Film and Ocular Surface) International Workshop on Contact Lens Discomfort, the subcommittee on neurobiology reviewed the role of ocular inflammatory mediators in ocular pain and discomfort.³⁸ However, in our current study, we found no significant increase in DCs in the subbasal layer in patients compared to controls. Further, the subset of patients who had received anti-inflammatory treatment, such as steroids, in the past had continued to experience symptoms of light sensitivity despite anti-inflammatory therapy. Thus, while inflammation may be involved in the pathogenesis of photoallodynia, such as in the initial induction of nerve damage, the current data suggest that it is primarily the neurotrophic support through AST that resulted in the improvement in symptoms. Interestingly, several studies have shown that corneal epithelial DCs may contain melanin.³⁹ Thus, one could speculate that these cells may act by sensing light in the cornea and provide an initial stimulus for firing of the trigeminal nociceptors in response to light, as corneal DCs are frequently seen in close contact to injured nerve terminals.⁷

Physiologic responses of corneal nerves to injury have been described in detail by Belmonte et al.⁴⁰⁻⁴² Nerve injury results in release of pro-inflammatory neuropeptides, including substance P and calcitonin gene-related peptide (**CGRP**), from both injured nerves and healthy nerves due to antidromic propagation of nerve impulses from the site of injury. These neuropeptides then produce plasma extravasation and vasodilation and stimulate the release of pro-inflammatory cytokines that induce inflammatory cells around the injured nerve terminals. One important effect of the inflammatory cytokines is the neuromodulation of the ion channels concentrated in the trigeminal nerve endings⁴³ through mechanisms that include altered protein expression and ion channel trafficking.^{44,45} Thus, chronic inflammation produces permanent changes in nociceptive terminals and diminishes neurite outgrowth in dorsal root ganglion cells by augmenting sodium load and reversing the operation of the sodium-calcium exchanger, ultimately resulting in increased calcium influx

across cell membranes and thus axonal degeneration.⁴⁶ Further, this results in the increased firing rate of the nociceptors to all forms of stimuli, in respect to both frequency and amplitude. Similar to peripheral neuropathies, sensitization is a prominent feature of corneal nociceptors, i.e., repeated noxious stimuli lead to a decrease in the threshold.⁴⁷ In addition, removal of stimuli only transiently abolishes the response, with the discharge of impulses becoming irregularly sustained with a lower frequency, irrespective of the presence of stimulus.⁴⁰ This is the physiologic basis for the development of neuropathy, called “phantom cornea.”⁴⁸

Currently, the pathophysiology of corneal neuropathy and the associated photoallodynia are not well understood, given the very recent description of this entity and the very limited available literature. Whatever the mechanism, the process needs to explain how light can produce discomfort or pain. A number of putative mechanisms have been suggested: 1) trigeminal systems and 2) retinal systems, or 3) a combination of both.

Damage to trigeminal corneal nerves can occur in association with surgical procedures (e.g., keratorefractive surgery),⁵⁴ dry eye disease,⁵⁵ systemic neuropathic conditions such as diabetes,^{56,57} infections such as herpes zoster ophthalmicus,⁵⁸ toxic damage during chemotherapy, recurrent corneal erosions, and exposure to noxious fumes or radiation and infections.^{2,3} Both spontaneous pain and evoked pain exacerbated by pressure or cold may relate to damaged nerves, as has been well described in peripheral neuropathies, and the altered morphology of nerve fibers observed is thus fully consistent with a primary neuropathic condition.⁴⁹ Spontaneous pain arises from damaged small fibers,⁵⁰ and the exacerbation of pain may be due to altered/lowered membrane potential as a result of such mechanisms as increased sodium channel expression.^{51–53} Using functional magnetic resonance imaging, Moulton et al showed that exposure to light in a photophobic subject activates the trigeminal system.⁵⁹ Furthermore, through a series of experiments in rats, Okamoto et al have proposed that intense light activates a trigeminal nociceptive pathway via increased parasympathetic outflow.⁶⁰ They identified a population of nociceptive nuclei in the spinal trigeminal nucleus, which are activated by mechanical stimulation of the ocular surface.⁶⁰ Electrophysiological measures evaluating neuronal activity show an increased firing rate in response to light, suggesting a reflex circuit that results in the stimulation of trigeminal nociceptors via exposure to light.^{61,62} Another potential pathway could be present via the autonomic fibers that accompany the trigeminal nerve. These fibers have been shown to mediate symptoms of light sensitivity in various neurological conditions through the trigeminovascular and trigeminoautonomic reflexes.⁶³

The second possible pathway involves the retinal systems. Photosensitive retinal cells may contribute in part to light aversion.⁶⁴ Presence of photophobia in blind patients⁶⁵ and after optic nerve resection in rats⁶⁶ demonstrates that an intact optic nerve is not essential for sensitivity to light. Such photosensitive non-image forming cells have been directly implicated in photophobia in migraine, during which light increases the pain.^{66,67} The potential pathway could involve melanopsin-containing photosensitive cells in the retina and the iris, the intrinsically photosensitive retinal ganglion cells that detect light and bypass conventional pathways through the retina and optic nerve.⁶⁷

Combination of trigeminal and retinal systems is also a plausible mechanism, as inputs from the retinal photoreceptors and trigeminal afferents converge at the level of the thalamus.⁶⁸ A crosstalk between these converging fibers may cause the perception of ocular discomfort and pain with light. From the thalamus, these afferents then project into various areas of the somatosensory, visual, and association cortices, explaining the broadly distributed response to light stimuli. Dolgonos et al have suggested the presence of an intraretinal mechanism, acting through associational ganglion cells, that modulates the trigeminal system to produce the ocular discomfort associated with exposure to bright light.⁶⁶

Our study has several limitations, including the relatively small sample size. However, no epidemiological data are currently available on corneal neuropathy, and many cases likely remain undiagnosed. Nevertheless, our results achieved clear statistical significance. Another limitation is the retrospective nature of this study. Given the promising results of this pilot study for a disease without any current treatment options, future larger randomized trials are now warranted to confirm and expand our findings. Performing long-term randomized studies in this patient population will be challenging, given the nature of the disease and severe impact on the quality of life.

We have used patient-reported severity and impact on quality of life as the parameters for severity of the condition. As photoallodynia is a very recently described entity, there are no established and validated objective tests for it. For future prospective studies, objective tests such as glare or contrast sensitivity testing, light scatter, corneal topography, and Fourier's analysis could be used as potential measures. Another limitation of this retrospective study is the lack of availability of corneal esthesiometry and correlation of corneal sensation with nerve density, which should be included in future prospective studies. However, as Belmonte et al have demonstrated before, abnormal nerve function following nerve injury may be associated with decreased sensation.⁴⁸ Hence, in patients with corneal neuropathy, sensitivity measurement may not correlate with nerve morphology as in normal subjects. Finally, our study has a relatively short follow-up time, and it is unclear if the outcomes and corneal nerve changes are retained long-term or are potentially reversible when the treatment with AST is discontinued. Future prospective studies on the use of AST for corneal neuropathy with a larger sample size and longer follow-up will likely provide more definitive answers on the long-term effects of AST on corneal nerves.

In conclusion, treatment with AST produces significant improvement in both patient-reported severity of symptoms of photoallodynia and recovery of corneal subbasal nerves as shown by IVCN. AST is easy to prepare, has limited side effects, and is a highly effective treatment for symptoms of corneal neuropathy, which have previously been severely undertreated and underdiagnosed.

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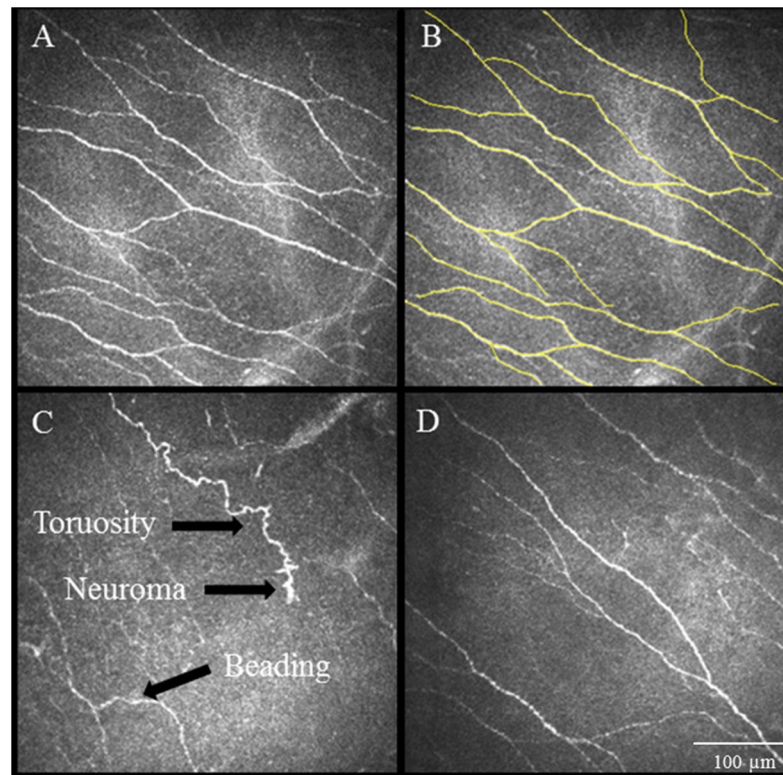


Figure 1. IVCM images obtained at the level of the corneal subbasal nerve plexus demonstrate nerve alterations in patients of corneal neuropathy. A. Normal corneal subbasal nerve plexus. B. Nerve tracings using Neuron J to quantify the density of nerves. C. IVCM images showing significantly decreased corneal subbasal nerve plexus at baseline, prior to autologous serum tears therapy. Note the decrease in length and number of nerves, increased tortuosity, reflectivity, beading and formation of neuromas. D. IVCM image showing post-treatment findings of the patient shown at panel C. Note improvement in the plexus, still abnormal as compared to normal controls. Size bar=100μm.

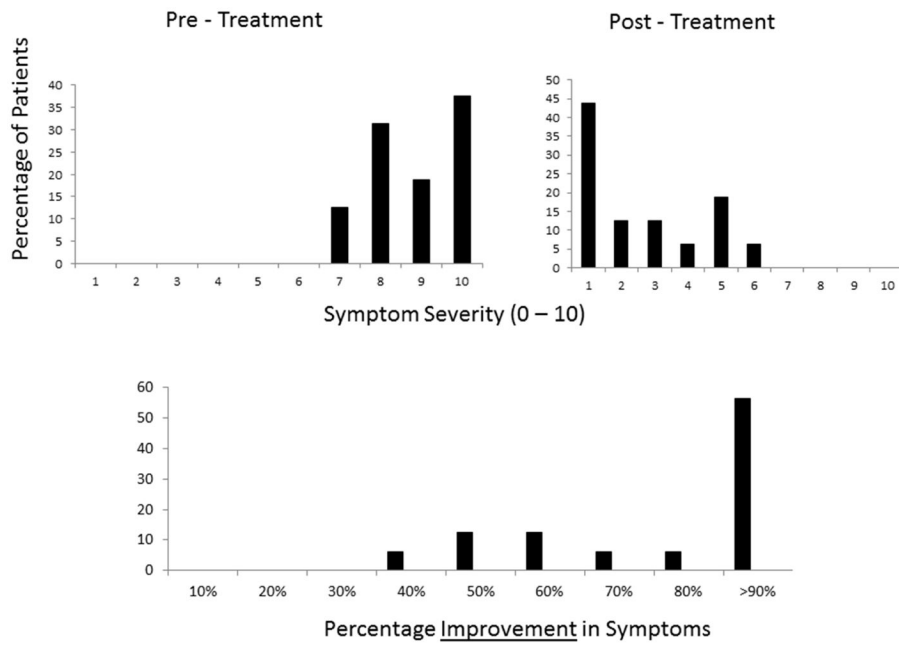


Figure 2. Severity of symptoms pre- and post-treatment and improvement in symptom severity post-treatment.

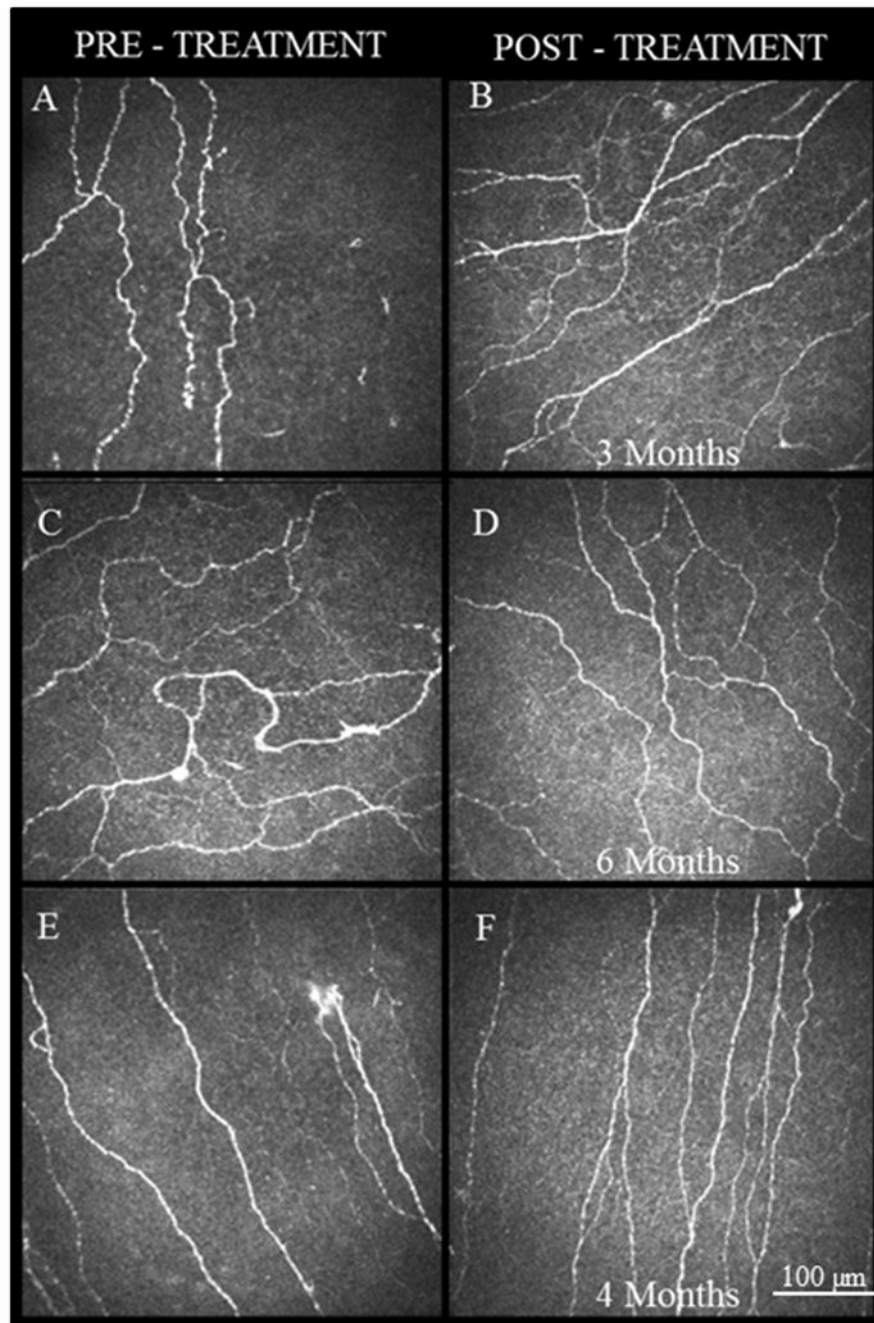


Figure 3.

IVCM images obtained at the level of the corneal subbasal nerve plexus demonstrate the improvement in the nerve density and morphology following treatment with autologous serum tears. A, C, E. Images at baseline showing increased reflectivity and tortuosity of nerves with presence of beading and neuromas. B, D, F. IVCM images post-treatment corresponding to same patients as A, C, E. Images after 3, 6 and 4 months, respectively, showing increased nerve density and decreased reflectivity, tortuosity, beading, and neuromas.

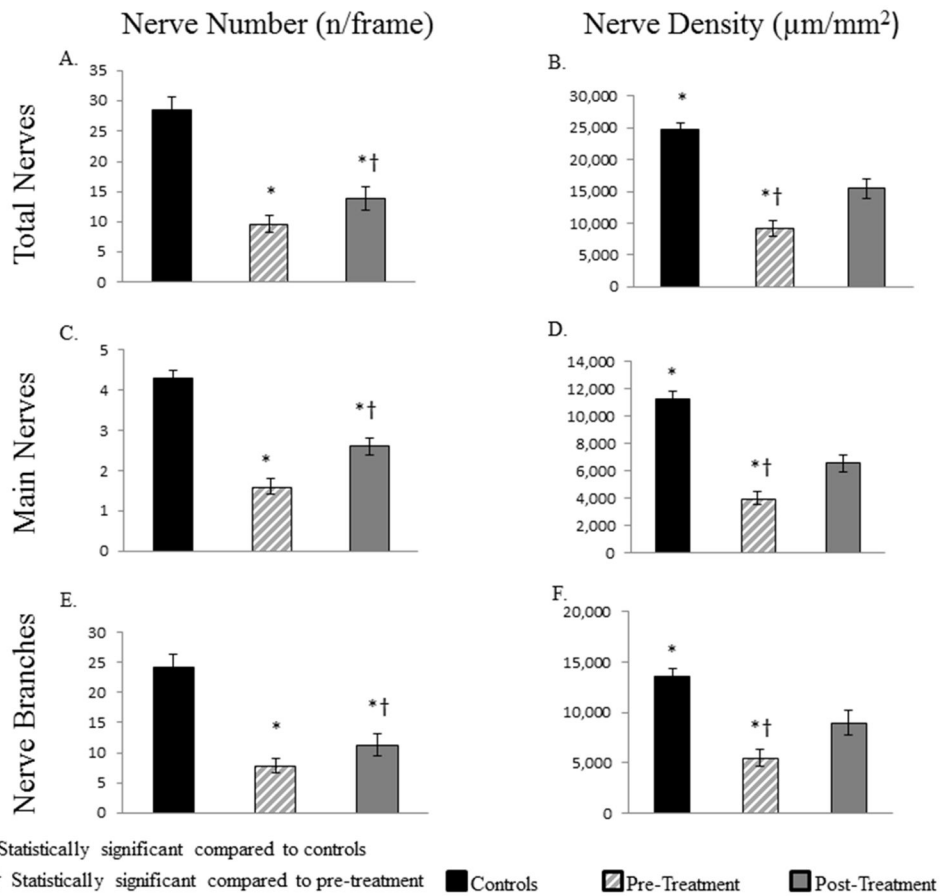


Figure 4. Comparison of subbasal nerve plexus density in patients suffering from corneal neuropathy, controls, and following treatment with ASTs. Bar graphs show corneal nerve alterations in pre-treatment and post-treatment eyes in patients and in the control group. A. Total number of nerves. B. Length of total nerves. C. Number of main nerve trunks. D. Length of main nerve trunks. E. Number of nerve branches. F. Length of nerve branches. * $P < .001$, compared to the control group by analysis of variance, † $P < .001$, compared to the pre-treatment group by paired *t*-test.

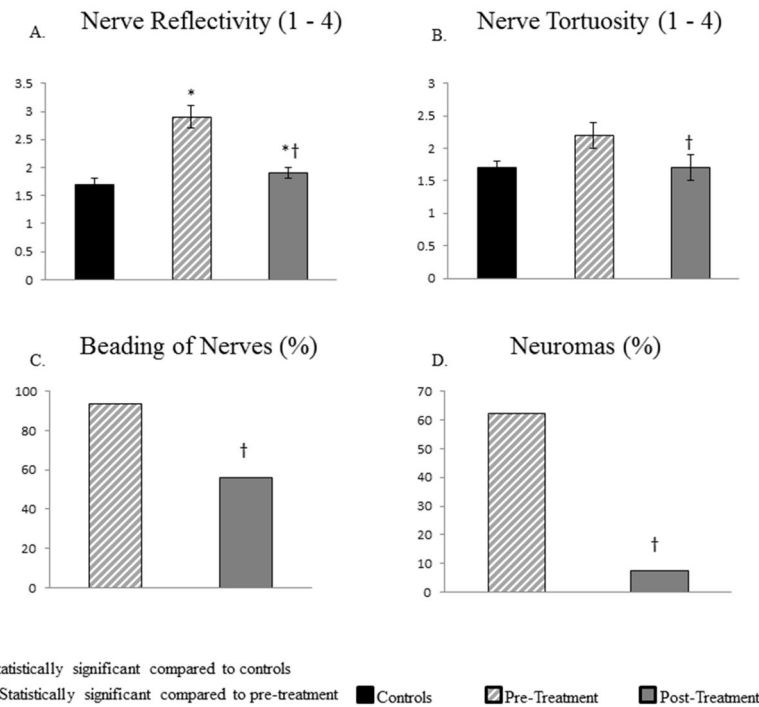


Figure 5.

Comparison of the subbasal nerve plexus morphology in patients suffering from corneal neuropathy, controls, and following treatment with ASTs. Bar graphs showing corneal nerve alterations in pre-treatment and post-treatment eyes in patients and in the control group. A. Reflectivity of nerves. B. Tortuosity of nerves. C. Beading of nerves. D. Neuromas. * $P < .001$, compared to the control group by analysis of variance, † $P < .001$, compared to the pre-treatment group by paired t-test.

Table 1
Demographic Data of Reference Controls and Patients with increased light sensitivity

	Controls	Patients	P value
No. of Patients	16	16	
Age (years)	56.4 ± 3.1 (range 25–74)	61.8 ± 4.4 (range 27–88)	P = 0.6
Gender (female/male)	10/6	10/6	P = 0.4

Values are mean ± standard error of mean unless otherwise specified.

Table 2

Clinical Features

Pt no.	Symptom duration	Activities affected	Impact	Underlying mechanism	Severity of photoallodynia (0/10)		Initial patient- reported symptom improvement (months)
					Before treatment	After treatment	
1	1 yr	TV, Reading	Eyes closed at all times	DES	7	0	8
2	3 yrs	Reading, driving	SDEI; Social impairment	DES	8	3	3
3	3 yrs	TV, Comp., driving	SDEI	DES	10	4	3
4	6 mths	TV	Social impairment	Radiation keratopathy	8	0	3
5	6 mths	Reading	SDEI, dropped out of school	DES	10	4	7
6	3 yrs	Reading, driving	Social impairment	DES	8	0	3
7	5 yrs	TV, Reading	SDEI, lost job	MGD	9	0	1
8	1 yr	Reading, Driving	SDEI, lost job	UV exposure	8	0	3
9	2 yrs	TV, Reading	SDEI	DES	10	1	1
10	1 yr	TV, Comp, Driving	SDEI	DES	10	5	6
11	4 yrs	Reading, Driving	Social impairment	DES	9	0	4
12	1 yr	Reading	SDEI	LASIK	8	4	2
13	5 mths	Walking around	SDEI	Radiation keratopathy	9	2	6
14	1 yr	TV, Comp	SDEI, lost job	CE, ABMD	10	2	3
15	9 mths	Reading, Driving	SDEI; Social impairment	LASIK	7	0	4
16	8 mths	Walking around	SDEI, lost job	DES	10	1	1

CE = Cataract Extraction; Comp. = Computer Use; DES = Dry Eye Syndrome; MGD = Meibomian Gland Dysfunction; UV = Ultraviolet; LASIK = Laser-Assisted in situ Keratomileusis; ABMD = Anterior basement membrane dystrophy; mths = months; OS = left eye; OU = Both eyes; SDEI = Sunglasses dependent even indoors; TV = Watching television; yrs = years

Corneal Subbasal nerve parameters in controls and eyes with photoalloidymia before and after treatment with autologous serum tears

Table 3

IVCM Parameter	Controls	Patients	
		Pre-Treatment	Post-Treatment
No. of total nerves (n/frame)	28.6 ± 2	9.6 ± 1.4*	13.9 ± 2*†
Total nerve length (µm/mm ²)	24,714 ± 1056	9,208 ± 1264*	15,451 ± 1595*†
No. of main nerve trunks (n/frame)	4.3 ± 0.2	1.6 ± 0.2*	2.6 ± 0.2*†
Length of main nerve trunks (µm/mm ²)	11,214 ± 602	3,958 ± 460*	6,525 ± 601*†
No. of nerve branches (n/frame)	24.3 ± 2	7.9 ± 1.2*	11.3 ± 1.9*†
Length of nerve branches (µm/mm ²)	13,519 ± 807	5,483 ± 838*	8,925 ± 1228*†
Tortuosity (0-4)	1.7 ± 0.1	2.2 ± 0.2	1.7 ± 0.2†
Reflectivity (0-4)	1.7 ± 0.1	2.9 ± 0.2*	1.9 ± 0.1*†

All values are reported as mean ± Standard error of mean unless otherwise specified.

* Statistically significant as compared to controls, p < 0.0001

† Statistically significant as compared to pre-treatment.