

LIQUID NITROGEN CRYOTHERAPY FOR SURFACE EYE DISEASE (AN AOS THESIS)

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ABSTRACT

Purpose: To evaluate the effects of new treatments with liquid nitrogen cryotherapy on some external eye conditions.

Methods: In this retrospective case study, 6 separate series from a single tertiary care referral center practice are described. Liquid nitrogen cryotherapy was used to treat conjunctival amyloidosis, primary pterygia, recurrent pterygia, advancing wavelike epitheliopathy (AWLE), superior limbic keratoconjunctivitis (SLK), and palpebral vernal keratoconjunctivitis (VKC). The main outcome measure was the resolution of the disease process after treatment.

Results: Four patients with primary localized conjunctival amyloidosis were treated with liquid nitrogen cryotherapy. Two of them had recurrence of the amyloidosis, which cleared with subsequent treatment. Eighteen patients with primary pterygia had excision and cryotherapy with 1 recurrence. Of 6 subjects who presented with recurrent pterygia, 4 had a second recurrence after excision and cryotherapy. In 5 patients with AWLE, the condition resolved within 2 weeks without recurrence or the need for subsequent cryotherapy. Four patients with SLK were treated with liquid nitrogen cryotherapy. Disease recurred in 2 patients and 3 of 7 eyes, although subsequent cryotherapy eradicated SLK in all cases. Two patients and 3 eyelids with palpebral VKC were treated with liquid nitrogen cryotherapy. VKC recurred in all cases.

Conclusions: Liquid nitrogen cryotherapy to the surface of the eye is effective in treating AWLE, and SLK. Excision followed by cryotherapy is successful in treating conjunctival amyloidosis and primary pterygia. Liquid nitrogen cryotherapy is unsuccessful in the treatment of recurrent pterygia and VKC.

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INTRODUCTION

Cryotherapy is used in ophthalmology to treat a variety of eye conditions. These include surface eye cancers,¹⁻⁴ cyclodestruction for uncontrolled intraocular pressure,⁵ retinopathy of prematurity,⁶ retinopexy in retinal detachments,⁷ periocular tumors, and trichiasis, to name a few.^{4,8-10} Cryotherapy may be preferable to other therapies in treating some eye diseases, as there are few postoperative adverse events and patients respond well to treatment without the long-term complications associated with radiation and cytotoxic therapies sometimes used in lieu of cryotherapy.¹¹ The experienced ophthalmologist can usually avoid serious complications by limiting the time that the cryogen is in contact with the surface of the eye.¹²

HISTORY OF CRYOTHERAPY

Cryotherapy has been called cryocautery, cryocongelation, cryogenic surgery, and cryosurgery. The known benefits of cryotherapy date back to 2500 BC, when the Egyptians used cold to soothe injuries.¹³ Hippocrates used cold to relieve swelling, bleeding, and pain.¹⁴ The first published report on freezing biologic tissue in a controlled fashion came from an Englishman, James Arnott (1797-1883), who was the senior physician at the Brighton Infirmary.¹⁵ Coincidentally, both Arnott and his brother, who invented the slow-combustion stove, became famous for their scientific work. Arnott was the first doctor to use freezing of tissue locally for the destruction of tumors. He used a salt and crushed ice mixture, which consisted of 2 parts crushed ice and 1 part sodium chloride. The equipment he designed for cryosurgery won him a prize medal at the Great Exhibition of London in 1851. The apparatus consisted of a waterproof cushion applied to the skin, 2 long flexible tubes to convey water to and from the cushion, a reservoir for the ice and water mixture, and a sump. Arnott used cryotherapy for multiple human maladies, including palliation of pain in breast cancer, uterine cancers, and treatment of some skin cancers. He also used his cold treatment for acne, neuralgia, and headaches, and was able to achieve temperatures of -24°C . Arnott also advocated using cold to numb the tissues prior to surgery. His procedure could be used in lieu of the anesthetic medicines being developed during his time. Ultimately, spreading his belief in cold as a targeted local anesthetic became his main goal in life. Although he was unsuccessful in this endeavor, his initial contribution to freezing therapy for human disease provided a key to future developments.¹⁶

Because the coldest temperature of the salt and ice mixture that Arnott could achieve was -24°C , advancements in cryotherapy would next come in the type of refrigerants used. Physicians were starting to realize that a rapid freeze with a colder cryogen was necessary to treat tumors effectively, and thus in the late 1800s advances were made in the liquefaction of gases. Cailletet¹⁷ was the first to demonstrate that oxygen and carbon monoxide could be liquefied under high pressure. At the French Academy of Science, on Christmas Eve 1877, this liquefaction of gas led Pictet¹⁸ to achieve similar results by liquefying oxygen using a mechanical refrigeration cascade. Within a few years, Von Linde was the first to commercially produce liquid air, in 1895. This was the key development in cold therapy's widespread acceptance.

Another key physician in the evolution of modern cryotherapy was Campbell White^{19,20} of New York. In 1899 he was the first to use the now commercially available refrigerants (liquefied gases) for medical care. He published reports on using liquid air for the treatment of lupus erythematosus, herpes zoster, nevi, warts, chancroid, varicose leg ulcers, carbuncles, and epitheliomas. Whitehouse²¹ furthered this research and used liquefied air on epitheliomata, lupus erythematosus, and vascular nevi on the skin.

Almost simultaneously with the development of liquefied gases, William Pusey of Chicago began using carbon dioxide snow. A steel storage container of carbon dioxide gas was used, from which the gas was allowed to escape causing a rapid expansion and a fall

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in temperature—the so-called Joule-Thomson effect. (In physics, the Joule-Thomson effect is a process whereby the temperature of a real gas is either decreased or increased by letting the gas expand freely at constant enthalpy, which means that no heat is transferred to or from the gas and no external work is extracted.²²) With Pusey's equipment, a fine snow was created and compressed into various shapes known as pencils. Pusey used these pencils to treat hairy nevi, warts, and lupus erythematosus as well as other skin diseases.²³ Hall-Edwards and Cranston-Low furthered this research with carbon dioxide snow, attaining skin surface temperatures slightly below -79°C .²⁴⁻²⁶

In the early 1900s there was debate on the best type of cryogen to use. Gold²⁷ and others decided that liquid air was far preferable; however, there was some difficulty in obtaining liquid air, whereas carbon dioxide snow was easier to come by. Irvine and Turnacliffe²⁸ expanded the uses of liquid air treatment to seborrheic keratosis, senile keratoses, lichen simplex, poison ivy dermatitis, and herpes zoster and warts. In fact, there was sometimes heated debate between carbon dioxide snow enthusiasts and liquefied air proponents, and Pusey and Irvine, in particular, exchanged less than friendly correspondence.²⁹

The development that allowed liquefied air to win out was the that of the vacuum flask by Dewar in 1898.^{17,30} This vacuum flask solved the problem of transportation and storage, and it is basically the same storage apparatus used in modern medicine for storage of refrigerants. Dewar's invention consisted of 2 walls of glass with a vacuum in between.²⁹

Allington³¹ was the first to use liquid nitrogen in the treatment of skin lesions. This became the preferred gas because liquid oxygen is explosive and the 2 gases have similar boiling points: 182.9°C for oxygen and 195.6°C for nitrogen. Allington used a cotton swab dipped in liquid nitrogen to treat skin tumors. Unfortunately, there was loss of the heat sink potential with the transfer between the cotton swab and the skin. This method was insufficient for effectively treating skin tumors, especially malignancies.

Next on the scene was Dr Irving S. Cooper, an American neurosurgeon from New York. In 1913, he designed a liquid nitrogen probe that was capable of achieving temperatures of -195.6°C . He treated Parkinson's disease and other neurologic disorders by freezing the thalamus. He also treated inoperable brain tumors with liquid nitrogen cryotherapy.³² Because of these advancements and others by Zacarian,³³ handheld devices were developed, which facilitated one-handed cryospray and cryoprobe procedures.

The research of Zacarian is the basis for much of the cryotherapy performed today, not only in dermatology but also in gynecology and ophthalmology.³⁴ He started using liquid nitrogen copper probes that allowed tissue freezing to depths of up to 7 mm. He extensively contributed to our current understanding of the benefits of liquid nitrogen cryotherapy and developed equipment to facilitate cryosurgical procedures. Ultimately, the research of liquid nitrogen cryotherapy in Europe came about because of the donation by Zacarian of a handheld cryosurgical unit to the Oxford dermatology department in the 1970s. Oxford became the center of cryosurgical research for Britain and Europe.³⁵

Liquid nitrogen cryotherapy spread to multiple specialties from that point forward. Dermatology research advanced significantly in the treatment of benign and malignant tumors of the skin. Neurosurgeons were able to perform liquid nitrogen-assisted transsphenoidal hypophysectomy. Liquid nitrogen treatment of oral and cervical cancers, cryosurgery to the uterus, and other advances took place.¹⁶

Currently, liquid nitrogen is by far the most popular cryogen. Carbon dioxide (-79°C) is still used worldwide because of relatively easy storage; however, because of its higher boiling point, it is suitable only for treating benign conditions. Similarly, nitrous oxide (-89°C) is favored by some gynecologists and oral surgeons, but only for benign lesions. Freon (-29.8°C to -40.8°C) has also been used to treat benign tumors. The standard of care in dermatology, oral surgery, otolaryngology, neurosurgery, and gynecology is liquid nitrogen cryotherapy for malignancies.^{16,29,35} Curiously, this has not translated to the subspecialty of ophthalmology, where multiple cryogens are used in lieu of liquid nitrogen for tumors on and around the eye.

ADVANCEMENTS IN CRYOTHERAPY WITHIN OPHTHALMOLOGY

Cryotherapy of the eye was first performed by Bietti in 1933. He reported on the use of cryogenics to produce a thermal chorioretinitis to seal a retinal hole. The technique was to use a metal probe, precooled in a mixture of carbon dioxide and acetone, and apply the probe to the outer wall of the eye overlying the retinal hole.³⁶ Soon thereafter, Deutschmann used cryosurgery in the form of solid carbon dioxide probes applied in the same fashion.³⁷ Surprisingly, these techniques were not adopted by ophthalmology for many years to come, and the research was largely ignored. Not until 1961 did cryosurgery reemerge within the subspecialty of ophthalmology. Krwawicz³⁸ developed a metal probe, which he immersed in a mixture of alcohol and solid carbon dioxide. After allowing enough time to lower its temperature, the probe was dried and applied to the moist anterior surface of the cataract to produce a firm attachment, and then withdrawn from the eye with the cataract attached to the tip of the probe. Improved cryoextraction techniques were achieved by Amoils,³⁹ who developed a liquid nitrogen probe that achieved cooling by expansion (the Joule-Thomson effect). He performed cataract extraction successfully, but cooling was slow and temperatures were not low enough for tumor work. Over the next few years, this technique of cryoextraction of cataracts was adopted by many ophthalmologists around the world.⁴⁰

Around this time, advances in treatment of retinal detachments and retinal tears were made with cryotherapy. Cryoretinopexy was done with various types of cryoapplicators. Two pioneers were Kelman⁴¹ and Bellows,⁴² who created retinal cryopexy instruments for the treatment of retinal tears and for cryoextraction. Schepens,⁴³ Lincoff,⁴⁴ and others were key figures in the advancement of cryoretinopexy surgical techniques and in using cryoretinopexy with scleral buckling for retinal detachments.³⁴

In most instances, solid carbon dioxide was utilized to achieve a temperature of -50°C to -60°C . Application of a cryoprobe to the sclera for 5 seconds was shown to create a white area in the underlying retina, and this cryotherapy could seal retinal tears and holes. These experiments were first achieved using animal models and then in humans.^{41,44}

After popularization of cryotherapy for cryoretinopexy and cryoextraction, cryotherapy, with different cryogens, has been used to

treat various eye diseases with frequent success. Numerous eye diseases are now treated with cryotherapy. Some conditions amenable to cryotherapy and of current interest are retinopathy of prematurity,^{6,45} uncontrolled intraocular pressure,⁵ retinal detachment,⁷ retinal capillary hemangiomas,⁴⁶ intraocular retinoblastoma,⁴⁷ trichiasis in ocular cicatricial pemphigoid,¹⁰ herpes keratitis,⁴⁸ and multiple types of eyelid and eyeball tumors.⁴ Also of interest are the historical advances in keratomileusis and lamellar corneal grafting techniques using a cryolathe and carbon dioxide snow put forward by Barraquer⁴⁹ and Kaufman,⁵⁰ respectively.

In 1972, Zacarian⁵¹ published the first series of cases ever described of liquid nitrogen cryosurgery around the eye and orbit for tumors. Based on his initial studies, Fraunfelder and colleagues became interested in the cryosurgical treatment of ocular and periocular squamous cell carcinomas, first in cattle (in which these tumors are histologically and clinically similar to human squamous cell cancers)⁵²⁻⁵⁴ and then in humans.^{4,55} Seminal papers on the treatment of surface eye tumors followed soon thereafter.^{2,3,56}

Liquid nitrogen cryotherapy for skin tumors, both benign and malignant, is the standard of care within the subspecialty of dermatology.⁵⁷⁻⁵⁹ This is not the case in ophthalmology, as few ophthalmologists use liquid nitrogen as the cryogen of choice for surface eye tumors.^{4,13,55,60,61} This is probably because of a lack of familiarity with the use and storage of liquid nitrogen and because ophthalmology training programs and fellowships at most institutions do not use this cryogen. The result is that, never having been exposed to it during their training, most ophthalmologists do not know when or how to use liquid nitrogen to treat eye disease.

CRYOGENS

Ophthalmologists who do use cryotherapy primarily use freon (-29.8°C to -40.8°C), nitrous oxide (-88.5°C), or solid carbon dioxide (-79°C) rather than liquid nitrogen (-195.6°C).³⁵ The boiling point of liquid nitrogen is by far the lowest of the available cryogens used in medicine, and it is therefore the most effective in cell destruction where a rapid freeze is associated with destruction of cells *in vivo*.⁶² It is well established in the dermatology literature that a rapid freeze of skin tumors with liquid nitrogen is much more effective than a slow freeze (using alternative cryogens) in eradicating tumor cells.^{8,57-59,63-65} This is true in the eye as well, where a number of case series have shown the tumoricidal nature of liquid nitrogen.^{2,3,56,66}

Within ophthalmology, the method of cryotherapy and types of cryogens used in the treatment of eye disease are not standardized. The practice of ophthalmic cryotherapy will most likely be based on the type of training the ophthalmologist was exposed to during residency and fellowship years. In addition, the physiochemical and biologic effects of the different cryogens on the ocular tissues are not fully elucidated.

SAFETY OF CRYOTHERAPY WITH LIQUID NITROGEN

Not all pathogenic organisms are destroyed completely by exposure to liquid nitrogen or other cryogens. In fact, cryogens such as liquid nitrogen, with very low boiling points, allow for an excellent rate of survival when employed for long-term storage of viruses and microorganisms. Using a cryoprobe, which is a closed system, involves no risk of contamination, as there is no exposure of vapor or sprays arising from the cryogen. However, with an open system such as a liquid nitrogen cryospray, precautions must be taken to prevent contamination from the freezing agent. This is achieved by passing the liquid nitrogen through a cryogenic filter when the liquid nitrogen tank is being filled. Examples of cryogenic filters include the Gelman filter and Millipore filter. Both of these consist of a glass prefilter and a 0.2- μm membrane filter combined in a single holder (Gelman Instrument Company, Ann Arbor, Michigan; Millipore Corporation, Billerica, Massachusetts). Brymill also sells a cold filter for use in when transferring the liquid nitrogen from the liquid nitrogen tank to the handheld cryotherapy unit (Brymill Cryogenic Systems, Ellington, Connecticut). That these filtration methods prevent contamination has been confirmed by repeated commercial and hospital bacteriologic tests of the cryogens, which have been consistently negative for bacteria, fungi, and molds.^{67,68,34}

Most complications from ocular cryotherapy with liquid nitrogen are related to surgeon inexperience and prolonged contact of a cryoprobe or cryospray with the surface of the globe. Many times, a novice cryosurgeon is unable to break the contact of the cryoprobe with the surface of the globe, leading to an overfreeze of the tissue. There is no heating element, since a rapid thaw markedly decreases the cytodestructive force of the freeze. The most common complications from cryotherapy are transitory uveitis, temporary chemosis, subconjunctival hemorrhage, corneal endothelial damage from deep freezing, possible paralysis of extraocular muscles from cryotherapy over muscle insertion sites and resultant destruction of nerve or muscle, and sector iris atrophy.¹¹ Rarely, there have been reports of scleral melting after liquid nitrogen cryotherapy.¹² Even though the above-mentioned adverse events rarely have long-term consequences, cryosurgery with liquid nitrogen is not a benign procedure and it requires practical experience. Beginning cryosurgeons will need to observe and learn when and how to treat surface eye tumors or other surface eye diseases prior to performing procedures themselves.

PHYSIOCHEMICAL EFFECTS OF CRYOTHERAPY

The mechanism behind how liquid nitrogen works is multifactorial and not fully elucidated. Some effects are well known, such as ischemia through the destruction of small-caliber blood vessels, ice crystal formation inside cells leading to cell wall rupture, denaturing of lipid-protein complexes, osmotic stress, vascular stasis leading to the loss of cellular blood supply, tissue necrosis, and the buildup of toxic concentrations of solutes inside cells, leading to cell death.^{60,69} The latter is explained by cell dehydration, which occurs when water is withdrawn to make the ice crystals. This dehydration inside the cell leads to cellular pH changes and the formation of a toxic concentration of salts in the cell. Slow thawing produces a longer exposure to this toxic substrate concentration and leads to recrystallization in cells, which increases cell destruction.⁶² Cellular apoptosis after freezing injury is another effect of cryosurgery.⁷⁰

The thaw phase of cryotherapy may be more important than the freeze phase in cell destruction. In essence, cell death is best

achieved by a rapid freeze (ice crystal formation, cellular ischemia by small-caliber blood vessel destruction) and a slow thaw (cell dehydration and a buildup of toxic substrates). The effect is enhanced by repeat freeze-thaw cycles, usually performed 2 or 3 times. The depth of freeze is related to the contact time: the longer the application, the deeper the freeze.⁶⁹

Which of the aforementioned events leads to cell death during cryosurgery depends on 4 main factors:

1. The nature of the cryogen employed, the method and length of its application, and the number of freeze-thaw cycles.
2. The type of cells being frozen. Melanocytes are the most sensitive, hence the risk of depigmentation of skin after cutaneous cryosurgery. Collagen is the most resilient tissue, and cartilage necrosis is extremely rare. Because of this, cryosurgery is particularly suitable in areas where maintenance of elasticity and function are important, ie, around the ear, around and on the eyes, and the nose.⁷¹⁻⁷³
3. The water content and vascularity of the tissue.
4. The rate of thaw.⁶⁹

Taking these points into account, some key theories regarding cryotherapy and its effect on tissues are important to understand. The ability of a cryogen to freeze tissues is related to its effectiveness in removing heat from the tissues. This is called the capacitance of the heat sink. The capacitance of a given heat sink is predetermined by its boiling point. The lower the boiling point, the greater the capacitance of the heat sink. Liquid nitrogen, with a boiling point of -195.6°C, has a very large capacitance as a heat sink and therefore has great potential to freeze tissues.

In addition, the ability of a cryogen to freeze depends not only on its heat sink capacitance, but also on the method by which it is applied to biologic tissue. If a thermocouple is placed within a tissue at a location next to the application of a cryoprobe, a rapid and precipitous temperature fall will be appreciated in the beginning. This will be followed by a slow fall in the temperature until a point is reached where the temperature equilibrates despite continuous cryogen application. This is because the cryogen acts as a heat sink while the tissues, reheated by blood vessels, resupply heat. The heat sink essentially loses efficiency with distance so that it is less effective in removing heat from the tissues at increasing distances from the cryoprobe application point. Eventually, a point is reached when heat is being renewed as fast as it is being supplied, a steady-state condition occurs, and the final temperature remains constant.⁷⁴

Another effect of cryotherapy is the freezing of extracellular fluid, which occurs when tissue is cooled slowly. Since ice is composed of pure water, this process of extracellular freezing removes water from the extracellular fluid and concentrates the remaining solutes. The freezing of pure water lowers its vapor pressure. In order to maintain thermodynamic equilibrium, the nonfrozen extracellular solution must continuously concentrate its solutes so that the vapor pressure of the solvent water is depressed to that of frozen water. At the same time the extracellular water is forming ice, the intracellular water is cooling below its freezing point but not forming ice crystals. This is called supercooling. The vapor pressure of supercooled water is higher than that of ice, and the cell membrane is permeable to water but not to ice crystals. The supercooled water will tend to flow out of the cell and freeze externally. The net result of this process is cellular dehydration and solute concentration. The combination of cell dehydration and exposure to concentrated solutes is probably what kills cells at slow thawing rates.⁷⁵

Another mechanism, and a more effective route to inducing cell death, is rapid freezing. In this instance, intracellular water will not have sufficient time to diffuse out of the cell, and the intracellular ice crystals that are formed are relatively unstable owing to their high surface-to-volume ratio. The formation of intracellular crystals is not uniformly lethal to all cells; however, if the cells are thawed slowly, the crystals will be converted into larger, more stable crystals by a process known as recrystallization. These larger recrystallized intracellular ice crystals are highly destructive to the cell, though the exact mechanism of injury is unknown.⁷⁶⁻⁷⁸

Another way to think about cryoinjury is the 2-phase hypothesis. In the initial phase there is a rapid freeze and formation of intracellular and extracellular ice crystals. In the second phase, ischemic infarction occurs from the obliteration of the microcirculation within the ice ball of frozen tissue. This leads to an all-or-none lethal effect; no cell surviving the initial phase can survive this ischemic infarction. Added to this, numerous studies have shown that cryotherapy is more tumoricidal using multiple freeze-thaw cycles than with a single freeze-thaw cycle. The effect is cumulative, and the reasons for this are not entirely clear.⁷⁹

From research by Wilkes and Fraunfelder⁶⁹ on the principles of cryosurgery, 15 points seem to hold true for cryotherapy:

1. The ability of a cryogen to freeze is dependent on its ability to remove heat. This is determined by its boiling point; the lower the boiling point, the greater the capacitance as a heat sink.
2. The ice ball produced by a cryoprobe has thermogradients. The thermogradients become warmer as distance from the cryoprobe is increased.
3. Thermogradients are closer together with a rapid freeze and further apart with a slow freeze.
4. Intracellular ice formation is produced with a rapid freeze. This is considered more damaging than the formation of extracellular ice that is produced with a slow freeze.
5. There are two phases of *in vivo* cryoinjury: intracellular and extracellular ice formation and ischemic infarction.
6. A rapid freeze and a slow thaw produce the most cell death.
7. Cryotherapy produces hemorrhagic necrosis after freeze-refreeze applications to the ciliary body.
8. Multiple freeze-thaw cycles are more destructive for both normal and pathologic tissue than a single cycle.
9. The pathologic hallmark of cryotherapy is ischemic necrosis.
10. Edema occurs after cryosurgery and resolves over 1 to 2 weeks.
11. Infection is rare after cryosurgery.
12. Healing time after cryosurgery ranges from 3 to 6 weeks.
13. Large blood vessels are highly resistant to cryoinjury, whereas microvasculature is susceptible.

14. Peripheral nerves are sensitive to cryoinjury.
15. Melanocytes are sensitive to cryoinjury.
16. Destruction of hair follicles occurs with double freeze-thaw cycles to -20°C .

In 1965, cryosurgeons treated skin cancers to -25°C ; then it was thought that the optimal treatment temperature for skin malignancies was -35°C . Currently, the acceptable temperature is -50°C to -55°C .⁵⁹ This number has been determined by electrical/resistance monitoring systems. These have been used to show thorough destruction of tissues at -50°C or lower temperatures.⁸⁰

PHYSIOLOGIC CONSIDERATIONS OF CRYOSURGERY ON THE SURFACE OF THE GLOBE

When freezing skin tumors or eyelid tumors, the temperature of the underlying tissues can be monitored by inserting a thermocouple. This can be accomplished by anesthetizing the area with local injections of lidocaine and inserting the thermocouple near the cryoprobe application site. For cryosurgery on the surface of the eye, this is not practical. One cannot insert a thermocouple into the sclera or cornea of the eye without putting the patient at risk of long-term scarring, infection, or globe perforation. To overcome this obstacle, a series of experiments were undertaken by Fraunfelder and Wingfield.⁵⁶

Presented at an American Ophthalmological Society meeting in 1980, these findings described a microspatula-thermocouple that was placed, through a vertical incision, 2 to 4 mm into the cornea of human eye bank eyes or anesthetized dog eyes. A cryoprobe was then applied adjacent to the thermocouple. If the cryoprobe was placed on the surface of the globe for only 2 to 3 seconds, no drop in temperature was recorded on the microthermocouple, suggesting that this was a safe amount of time to apply a cold liquid nitrogen cryoprobe. In contrast, prior research by Fraunfelder and associates⁴ revealed loss of endothelial cells with freezing times approaching 5 seconds. A temperature of -25°C at the level of the endothelium will kill these fragile cells, and an ice ball of 5 mm or larger did lead to endothelial cell loss. Based on the data from these studies, a series of surface eye malignancies was treated with liquid nitrogen cryotherapy, taking care to keep the contact time of the cryoprobe to less than 3 seconds, and usually 1 to 2 seconds. Case series on eyelid basal cell carcinoma and squamous cell carcinoma were published; also treated were surface ocular squamous cell carcinoma and intraepithelial epithelioma. Other conditions initially treated were trichiasis, papillomas, actinic keratosis, reactive lymphoid hyperplasia, conjunctival inclusion cysts, and conjunctival papillomatosis.⁴ Case series have been published on the 3 main categories of surface eye malignancies: primary acquired melanosis and malignant melanoma,³ reactive lymphoid hyperplasia and conjunctival lymphoma,² and conjunctival intraepithelial neoplasia and conjunctival squamous cell carcinoma.⁵⁶ Using the techniques described above for liquid nitrogen cryotherapy of the ocular surface, complication rates were very low.

Most ophthalmic research on the effects of liquid nitrogen cryotherapy has addressed surface eye tumors and eyelid malignancies rather than other types of external eye disease.^{2-4,56} The effects of cryosurgery on the surface of the eye and eyelids should also translate to benign eye conditions with potential to affect vision.

METHODS

This study was approved by the Institutional Review Board of Oregon Health & Science University (OHSU), and all patients gave informed consent for surgery and for publication of research findings. All patients referred to Casey Eye Institute, OHSU, between January 2002 and December 2006 and diagnosed with the visually significant conditions of conjunctival amyloidosis, primary pterygia, recurrent pterygia, advancing wavelike epitheliopathy (AWLE), superior limbic keratoconjunctivitis (SLK), or vernal keratoconjunctivitis (VKC) were included if they underwent cryotherapy. Visual significance is defined as loss of best-corrected visual acuity or ocular discomfort to the point of interruption of activities of daily living. Two subjects with visually significant recurrent pterygia did not undergo treatment with liquid nitrogen cryotherapy, and one patient with visually significant SLK deferred cryotherapy treatment. The pterygia were treated with conjunctival autografts, and the SLK was treated with silver nitrate. Otherwise, any patient referred with these visually significant conditions consented to treatment with liquid nitrogen cryotherapy. Data were collected on each patient's age at the time of diagnosis and treatment, gender, race, diagnosis and laterality of eye disease, concomitant systemic disease, and oral or topical medications. Not all patients were treated with cryotherapy; for example, if the pterygium was not visually significant or the SLK was not causing symptoms, these conditions could be treated conservatively. Forty-one eyes of 41 patients had non-visually significant primary pterygia and were not treated with cryotherapy, but with conservative management. There were 4 eyes of 2 patients with SLK who were not deemed to have severe enough symptoms to undergo cryotherapy. All patients with conjunctival amyloid, AWLE, and vernal disease were treated with cryotherapy during this time period and had visually significant consequences from their eye disease. The number of subjects in each group included only those treated with liquid nitrogen cryotherapy. Follow-up is ongoing.

The above-mentioned eye diseases were chosen based on the expected biologic effect of liquid nitrogen cryotherapy on the disease. The effect of cryotherapy on small-caliber blood vessels and the toxicity of cryotherapy to cells leading to cell death led the author to consider this mode of treatment for conditions where targeted cell death and ischemia were desirable. Liquid nitrogen was chosen as the cryotherapy agent because its boiling point is the lowest of the gases used, and it will therefore have the most profound effects. A rapid freeze with a low boiling point cryogen is more toxic to living cells than a slower freeze, such as would occur with alternative cryogens.^{57-59,65}

The cryotherapy unit was a Brymill CRY-AC-3 liquid nitrogen container with a 300-mL capacity (Brymill Cryogenic Systems, Ellington, Connecticut) (Figures 1 and 2). Various spray tips and probes can be attached to the unit, depending on the disease and surface being frozen. Conical probes with tips ranging from 1 to 6 mm are available; spray tip apertures range from 0.04 inches (A tip) to 0.011 inches (F tip). The probes and spray tips are designed primarily for dermatologic and gynecologic surgery. The conical

probes in the range of 1 to 3 mm and spray tips of 0.016 inches (D tip) and 0.013 inches (E tip) tend to work best on the surface of the eye.^{81,82} Since the boiling point of liquid nitrogen is -195.6°C , allowing the cryoprobe tip to freeze until the frost around the probe liquefies (approximately 15 seconds) confirms that the lowest temperature of liquid nitrogen has been reached. It is more difficult to assume the temperature for the cryospray aperture use of liquid nitrogen, as the liquid is converted to gas as it comes out of the nozzle. However, as the tumoricidal temperature for malignancies is known to be -50°C and cell death for nonmalignant cells is as low as 25°C , the cryospray from liquid nitrogen should be sufficient if applied properly for the eye conditions described.³⁵ The nozzle of the cryospray unit is held very close to the area of disease being treated, as close as possible without touching the eye or eyelid in order to maximize the desired rapid freeze. The disease presentations and surgical techniques for treating each disease are described below.



FIGURE 1

Cryotherapy apparatus: Brymill liquid nitrogen unit with spray tip aperture attached.



FIGURE 2

Cryotherapy apparatus. Brymill liquid nitrogen unit with cryoprobe attachment.

CONJUNCTIVAL AMYLOIDOSIS

The patients presented with painless swellings of the conjunctiva or eyelids and, in some instances, pseudoptosis. Epiphora was a common complaint. Clinically, the conjunctival lesions were small pink-red or yellow-red nodules that were well vascularized (Figure

3, left). The palpebral conjunctiva and forniceal conjunctiva were primarily involved. All patients included here had primary localized conjunctival amyloidosis without systemic involvement and no antecedent ocular disease, such as chronic glaucoma or severe surface ocular disease. All patients were referred to their primary care providers for systemic workup, with particular attention to signs that might indicate plasma cell malignancy, rheumatoid arthritis, inflammatory bowel disease, or untreated familial Mediterranean fever. Patients were also questioned closely to determine if they had any history of familial amyloidosis. One patient (patient 4, Table 1) underwent magnetic resonance imaging of the brain and orbit to rule out a plasmacytoma. A biopsy was performed on all lesions to rule out malignancy and confirm the diagnosis of amyloidosis. Histopathology results showed acellular amorphous eosinophilic material with the characteristic staining with Congo-red of dichroic birefringence.



FIGURE 3

Patient with conjunctival amyloidosis (patient 3, Table 1). Left, Conjunctival amyloidosis involving the palpebral conjunctiva of the right upper eyelid. Right, Conjunctival amyloidosis after excision and cryotherapy to the right upper eyelid.

All subjects were outpatients who had local anesthesia with a combination of a single drop of topical proparacaine and subconjunctival or intratarsal injection of 0.5 to 1 mL lidocaine 1% with epinephrine. Cryotherapy was performed on the lesions, either directly (patient 1, Table 1) or after excisional biopsy to debulk the amyloid lesions (patients 2, 3, and 4, Table 1). Liquid nitrogen cryospray was applied using a Brymill D tip with a 0.016-inch aperture until lesion(s) appeared chalk white. This took approximately 2 to 3 seconds. Using a double freeze-thaw technique, the lesion was allowed to thaw (approximately 5 to 10 seconds) and the process was repeated. Erythromycin eye ointment was placed on the eye, and patients were instructed to place this in the fornix of the involved eye 4 times daily for 1 week. Patients were seen again after cryotherapy at intervals of 1 day, 1 week, 2 weeks, 3 months, 6 months, and yearly thereafter.

PTERYGIA

Patients referred to Casey Eye Institute with pterygia presented with symptoms of eye redness, foreign body sensation, tearing, photophobia, and/or ocular pain. Some patients had visual loss due to induced astigmatism or encroachment of the pterygium on the visual axis. Clinically, the pterygia looked like wing-shaped folds of conjunctival and fibrovascular tissue extending over the limbus and encroaching onto the superficial cornea (Figure 4, left).

If the pterygium placed the patient at risk of vision loss or was causing symptoms unacceptable to the patient, an excisional biopsy with adjuvant liquid nitrogen cryotherapy was performed. An avulsion technique was used to remove the pterygium, as described by multiple authors.⁸³⁻⁸⁵ All subjects were outpatients who had local anesthesia with a combination of a single drop of topical proparacaine and subconjunctival injection of 0.5 to 1 mL lidocaine 1% with epinephrine around the base of the pterygium. The conjunctival portion of the pterygium was first dissected forward to the corneoscleral limbus, and a 4-0 silk suture was tied around the base of the pterygium at the limbus. The pterygium was then avulsed by rapidly pulling on the 4-0 silk in the direction of the cornea. Cryotherapy, with a 2-mm cryoprobe attached to the Brymill unit, was then performed, with the tip of the cryoprobe in contact with the corneoscleral limbus for approximately 1 second. A double freeze-thaw technique was used, with approximately 30 seconds elapsing between freeze applications. After the cryotherapy, the conjunctiva was closed with 6-0 plain gut sutures up to the limbus. Histopathologic results revealed elastotic degeneration of collagen and the appearance of subepithelial fibrovascular tissue. Malignancy was also ruled out with pathologic examination. Patients were prescribed topical ocular corticosteroids and topical ofloxacin to be taken 4 times daily for 2 weeks, and instructed to return for follow-up visits at 1 day, 1 week, 2 weeks, 3 months, 6 months, and yearly thereafter.

TABLE 1. CASE REPORTS OF PATIENTS TREATED WITH LIQUID NITROGEN CRYOTHERAPY FOR CONJUNCTIVAL AMYLOIDOSIS

PATIENT	AGE	GENDER	LOCATION	SYSTEMIC INVOLVEMENT	EXCISION AT TIME OF CRYOTHERAPY	RECURRENCE	FOLLOW-UP DURATION (mo)
1	70	Male	Left upper eyelid palpebral conjunctiva	No	No	No	18
2	50	Male	Right eye temporal bulbar conjunctiva	No	Yes	No	26
3	60	Female	Right upper eyelid palpebral conjunctiva	No	Yes	Yes (14 mo)	29
4	57	Female	Right eye superior bulbar and upper eyelid palpebral conjunctiva	No	Yes	Yes (10 mo)	23

In cases 3 and 4, disease was re-treated within 2 weeks of recurrence. The conjunctival amyloidosis resolved after subsequent liquid nitrogen cryospray.

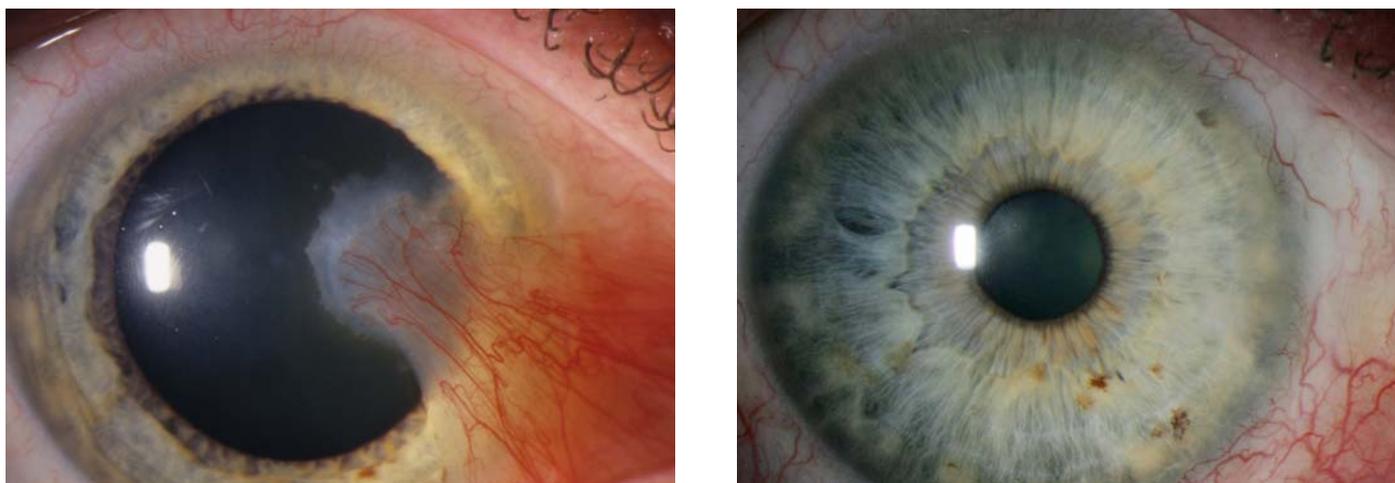


FIGURE 4

Patient with pterygium treated with excision and cryotherapy (patient 6, Table 3). Left, Pterygium involving the bulbar conjunctiva and cornea of the right eye. Right, Resolution of pterygium after excision and cryotherapy.

ADVANCING WAVELIKE EPITHELIOPATHY

Patients with AWLE presented with foreign body sensation or blurred vision. The disorder was diagnosed by the characteristic well-demarcated patches of coarse, irregular epithelium that appeared in recurrent waves extending from the superior limbus toward the visual axis (Figure 5, left). The waves were most easily seen by shining a broad slit-lamp beam tangentially across the surface of the cornea, or with sclerotic scatter revealing the distinct margins. Fluorescein dye produced a punctate staining pattern over the lesion. If

the AWLE was encroaching on the visual axis, liquid nitrogen cryotherapy was performed. In all cases, Papanicolaou smears of the surface epithelial cells were normal before cryotherapy was performed.

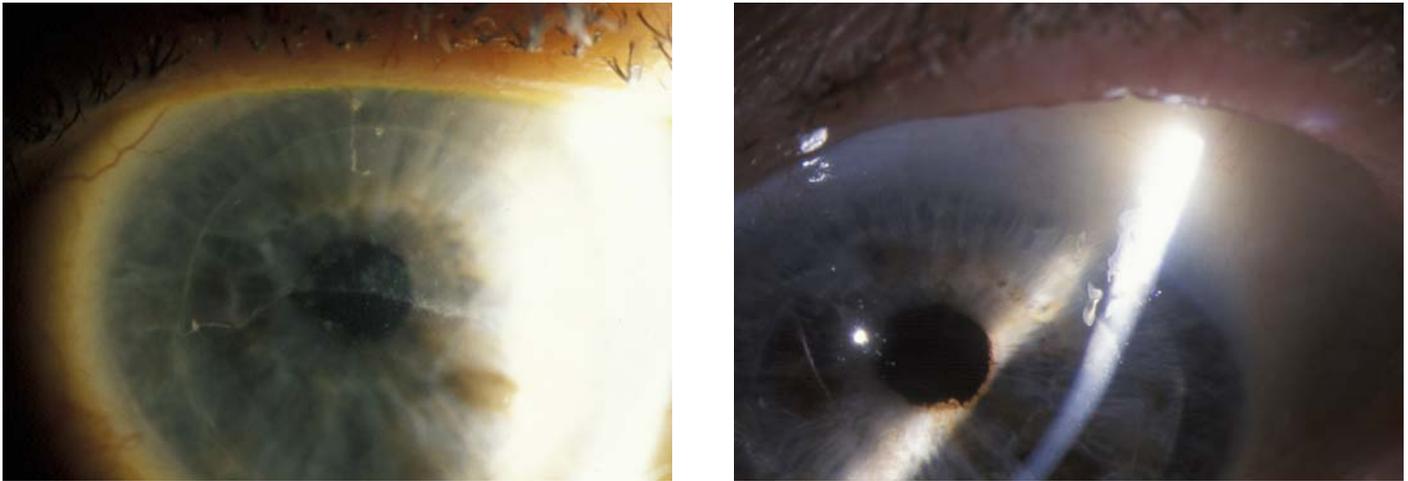


FIGURE 5

Patient with advancing wavelike epitheliopathy (AWLE). Left, AWLE encroaching on the visual axis (case 4, Table 4). Right, AWLE after cryotherapy (case 4, Table 4). (Photos reproduced with permission from Lippincott Williams & Wilkins. Fraunfelder FW. Liquid nitrogen cryotherapy of advancing wavelike epitheliopathy. *Cornea* 2006;25:196-198.)

All subjects were outpatients who had local anesthesia with a single drop of topical proparacaine. In addition, a Weck-cell sponge was soaked in proparacaine and held over the superior limbal area for 30 seconds for additional anesthesia. A D spray tip (0.016-inch aperture) was placed on the Brymill cryotherapy unit, and a double freeze-thaw was performed on the corneoscleral limbus and surface corneal epithelium in the 4 patients affected by AWLE. An adequate freeze was obtained when the surface cells appeared chalky white after application of the spray (approximately 1 to 2 seconds). The initial cryotherapy was followed by a slow thaw (lasting approximately 5 to 8 seconds after the frost disappeared), and then the second cryotherapy was performed in the same manner. No epithelial debridement was performed. After treatment, patients were seen again at 1 day, 1 week, 2 weeks, 1 month, 3 months, 6 months, and yearly thereafter. Subjects used ofloxacin eye drops 4 times daily for 3 postoperative days.

SUPERIOR LIMBIC KERATOCONJUNCTIVITIS

The diagnosis of SLK was based on one or more of the following disease presentations: unilateral or bilateral ocular burning, foreign body sensation, ocular pain, epiphora, photophobia, blepharospasm, and sometimes decreased vision. Some patients had mucus discharge and corneal filaments. On examination, the superior conjunctiva was found to be inflamed and red with fine punctate staining by rose bengal of the upper cornea, superior limbus, and adjacent conjunctiva (Figure 6). Filaments were sometimes present



FIGURE 6

Superior limbic keratoconjunctivitis (patient 2, Table 5).

at the superior limbus, with some patients exhibiting pseudoptosis. There was usually a fine papillary reaction of the upper eyelid palpebral conjunctiva as well (Figure 7). Patients who presented with symptoms and signs of SLK were tested for abnormalities in thyroid function.

FIGURE 7

Fine papillary reaction on palpebral conjunctiva in superior limbic keratoconjunctivitis SLK (patient 4, Table 5)..



If conservative management with artificial tears, topical ocular corticosteroids, or ocular lodoxamide was not beneficial, liquid nitrogen cryotherapy was performed using a Brymill E tip spray (0.013-inch aperture) with a double freeze-thaw technique. All subjects were outpatients who had local anesthesia with a single drop of topical proparacaine. In addition, a Weck-cell sponge was soaked in proparacaine and held over the superior conjunctiva and limbus area for 30 seconds for additional anesthesia. Care was taken to create a chalk-white appearance prior to the thaw, which usually required 1 to 2 seconds of freezing. The superior limbus and inflamed conjunctiva were treated in this manner, and the process was repeated after a thaw of 5 to 8 seconds. Following the procedure, patients used ofloxacin eye drops 4 times daily for 3 days. They were rechecked at posttreatment intervals of 1 day, 2 weeks, 3 months, 6 months, and yearly thereafter.

VERNAL KERATOCONJUNCTIVITIS

The diagnosis of VKC was based on the characteristic symptoms of ocular itching, tearing, mucus secretion, photophobia, and the presence of upper tarsal giant papillae (Figure 8), corneal shield ulcers (Figure 9), or gelatinous limbal infiltrates (Horner-Trantas dots). Patients treated with cryotherapy were those whose disease did not respond satisfactorily to topical treatment with cyclosporine, lodoxamide, olopatadine, sodium cromoglycate, topical corticosteroids, topical nonsteroidal anti-inflammatory drugs (NSAIDs), or artificial tears.

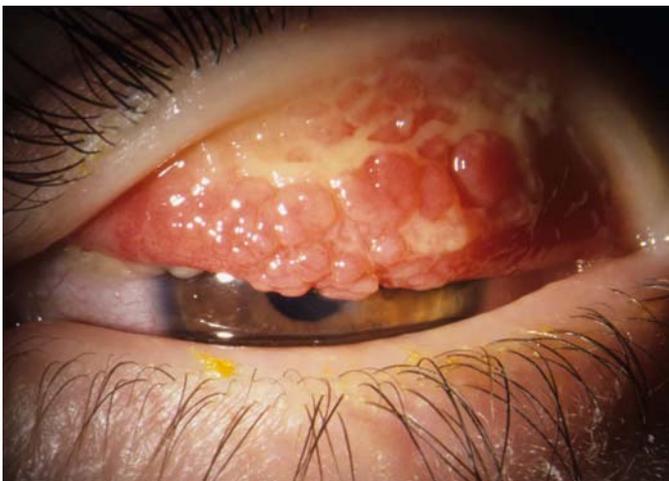


FIGURE 8

Giant papillary conjunctivitis in palpebral vernal keratoconjunctivitis (case 1, Table 6).



FIGURE 9

Corneal shield ulcer in vernal keratoconjunctivitis (case 2, Table 6).

One subject was an outpatient who had local anesthesia with a combination of a single drop of topical proparacaine with subconjunctival and intratarsal injection of 0.5 to 1 mL 1% lidocaine with epinephrine. The second subject was a 9-year-old boy who was placed under general anesthesia for bilateral cryosurgery. A 2-mm conical cryoprobe was held in contact with the giant papillary conjunctival reaction (giant papillary conjunctivitis) on the palpebral conjunctiva of the upper eyelid for approximately 1 second. This procedure was performed under both general anesthesia (in children) and topical anesthesia in the examination lane (in adult patients). Cryotherapy was performed twice, allowing 30 seconds of thaw time between freezes. Postoperatively, erythromycin eye ointment was applied topically, and patients were discharged with instructions to return at intervals of 1 day, 1 week, 1 month, 3 months, 6 months, and yearly thereafter.

STATISTICAL ANALYSIS

Variables are expressed as median. Statistical analysis for primary pterygia is demonstrated by a Kaplan-Meier curve.⁸⁶

RESULTS

CONJUNCTIVAL AMYLOIDOSIS

Four patients with primary localized conjunctival amyloidosis—2 men and 2 women with a median age of 58.5 years (range, 50 to 70 years)—were treated with liquid nitrogen cryotherapy (Table 1). Median length of follow-up was 24.5 months (range, 18 to 29 months). Patients 3 and 4 had posttreatment recurrence of conjunctival amyloidosis after the first treatment, at 14 and 10 months, respectively. The systemic workup seeking other causes of amyloidosis was negative in all patients. Every patient had an excisional biopsy to make the diagnosis of amyloidosis; however, only patient 1 underwent cryotherapy alone after the excisional biopsy. Patients 2, 3, and 4 had additional debulking of the conjunctival amyloidosis prior to liquid nitrogen cryotherapy at the base of the excision sites. Photographs of patients 2 and 3 before and after surgery with excision and cryotherapy are shown in Figures 3 and 10. Subjectively, the patients were satisfied with the surgical results, and objectively, the conjunctival amyloidosis was eradicated in all subjects after subsequent cryotherapy.

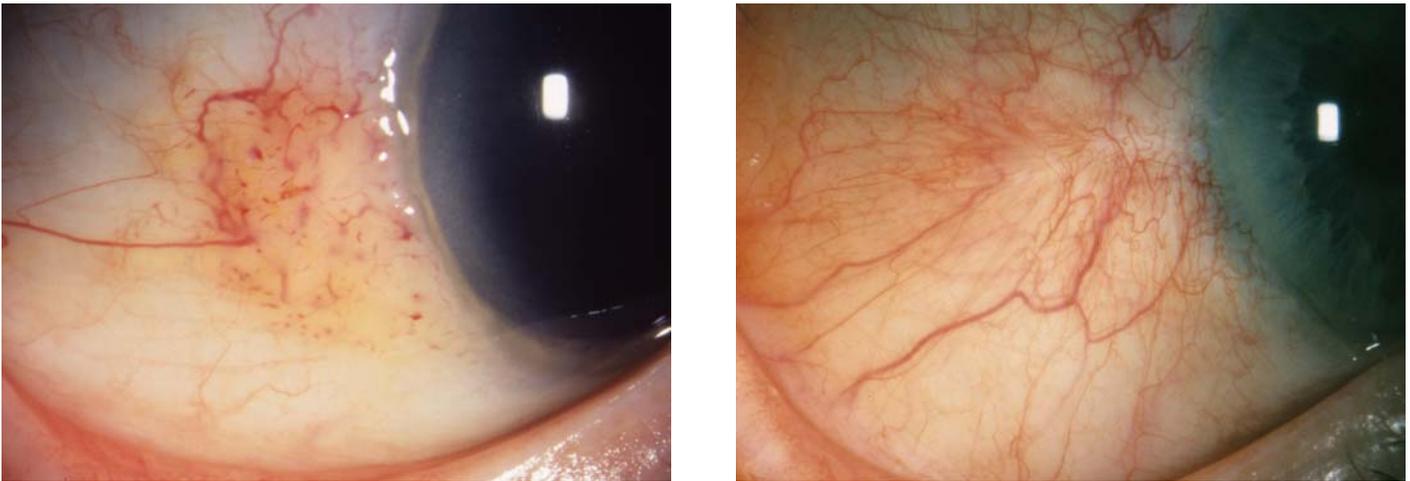


FIGURE 10

Patient with conjunctival amyloidosis (patient 2, Table 1). Left, Conjunctival amyloidosis involving the limbus of the right eye. Right, Resolution of amyloidosis after excision and cryotherapy.

PTERYGIA

Two groups of patients underwent liquid nitrogen cryotherapy: patients referred to Casey Eye Institute who had no prior surgery and those in whom pterygia had recurred after excision at another hospital without adjunctive therapy. Eighteen patients (18 eyes) who had no prior surgery had a median of 24.5 months follow-up (range, 10 to 56 months). The median age was 56 years (range, 36 to 74 years) with 11 males and 7 females. In this group, only patient 15 had a recurrent pterygium, which recurred at 9 months after surgery, giving a recurrence rate of 5.5% (Table 2; Figure 11).

In patients presenting with recurrent pterygia, the median age was 39 years (range, 31 to 67 years) with 3 men and 3 women and a median of 27 months follow-up (range, 13 to 80 months) (Table 3). Four of the 6 patients had recurrent pterygia, an average of 13 months (range, 2.5 to 42 months) after excision and cryotherapy. Four of 6 were Latino, and 3 of these 4 Latino patients had recurrence an average of 3.3 months (range, 2.5 to 4.5 months) after excision and cryotherapy.

Before-and-after photographs from both pterygium patient populations are included for patient 6 (Table 3) and patient 6 (Table 2) (Figures 4 and 12).

TABLE 2. CASE REPORTS OF PATIENTS TREATED WITH LIQUID NITROGEN CRYOTHERAPY AFTER PRIMARY PTERYGIUM EXCISION

PATIENT	AGE	GENDER	EYE	RACE	RECURRENCE	FOLLOW-UP DURATION (mo)
1	47	Female	Right	White	No	50
2	39	Female	Right	Asian	No	44
3	74	Male	Right	White	No	56
4	59	Female	Left	Asian	No	44
5	55	Female	Right	White	No	38
6	36	Female	Left	White	No	48
7	43	Male	Left	White	No	37
8	44	Male	Right	White	No	43
9	52	Male	Right	White	No	27
10	67	Male	Left	Asian	No	19
11	65	Male	Right	Latino	No	21
12	68	Male	Left	White	No	10
13	45	Female	Right	Latino	No	16
14	68	Female	Left	White	No	10
15*	64	Male	Left	White	Yes (9 mo)	19
16	37	Male	Left	White	No	20
17	62	Male	Left	White	No	14
18	57	Male	Right	White	No	22

*Patient was not treated again despite recurrence.

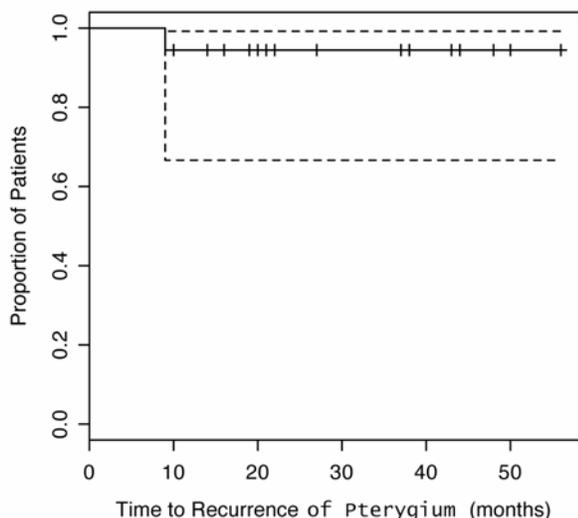


FIGURE 11

Cryotherapy for pterygium: Kaplan-Meier plot showing the proportion of patients vs time to recurrence in months. The dashed lines indicate the 95% confidence interval. The tick marks along the data line indicate points of censoring.

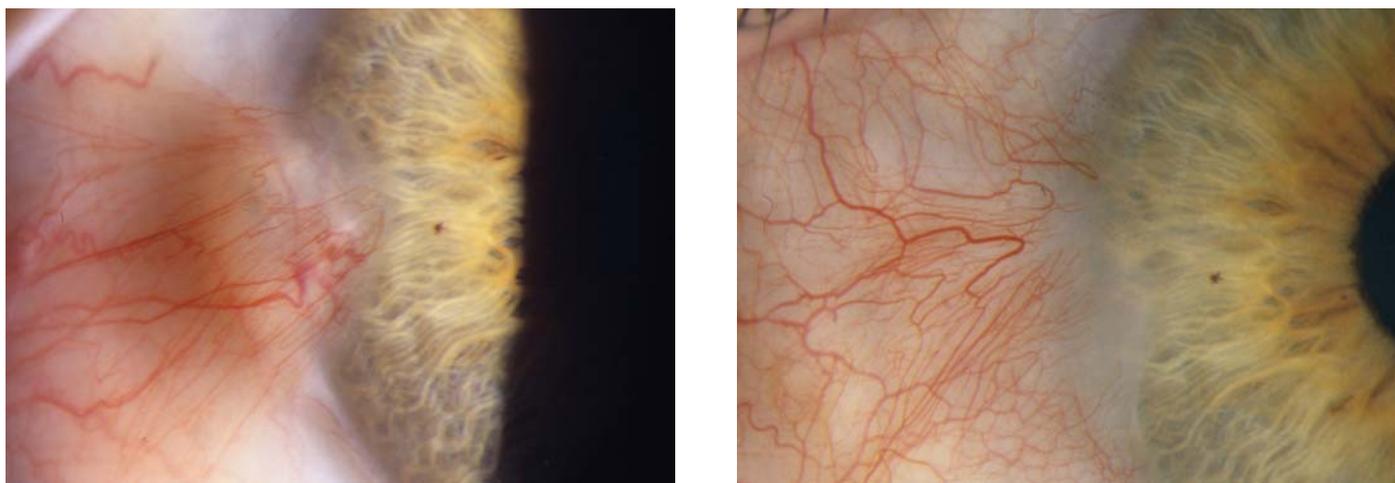
ADVANCING WAVELIKE EPITHELIOPATHY

In all 5 patients, median age was 77 years (range, 76 to 83 years), and AWLE resolved within 2 weeks without recurrence or the need for repeated cryotherapy after 1 year (Table 4). Median length of follow-up was 15 months (range, 12 to 18 months). Four of the 5 eyes were treated with eye drops for glaucoma, and 2 had a history of corneal transplantation. None of the patients in this series wore contact lenses. Visual acuity improved only slightly in all subjects, and antecedent eye disease, such as cataracts, glaucoma, and irregular astigmatism, limited the best-corrected visual acuity. There were no surgical complications as a result of treatment with liquid nitrogen cryotherapy spray. Figure 5 illustrates the case of patient 4 before and 1 year after cryotherapy. These case reports were published in 2006.⁸⁷

TABLE 3. CASE REPORTS OF PATIENTS TREATED WITH LIQUID NITROGEN CRYOTHERAPY AFTER EXCISION OF RECURRENT PTERYGIA

PATIENT	AGE	GENDER	EYE	RACE	RECURRENCE	FOLLOW-UP DURATION (mo)
1	40	Female	Left	Latino	No	35
2	38	Male	Left	Latino	Yes (4 ½ mo)	48
3	67	Female	Right	White	No	13
4	31	Male	Left	Latino	Yes (3 mo)	13
5	33	Female	Right	Latino	Yes (2 ½ mo)	19
6*	53	Male	Right	White	Yes (3 ½ yr)	80

*Only patient 6 had a second excision and cryotherapy, and the pterygium did not recur again

**FIGURE 12**

Patient with a pterygium (patient 6, Table 2). Left, Pterygium at corneoscleral limbus. Right, Resolution of pterygium after excision and cryotherapy.

SUPERIOR LIMBIC KERATOCONJUNCTIVITIS

Four patients, all female, with a median age of 64 years (range, 51 to 78 years) were treated with liquid nitrogen cryotherapy for SLK (Table 5). Three of 4 patients had bilateral disease and 2 had associated thyroid disorders. Resolution of symptoms and signs occurred within 2 weeks in all cases. The SLK recurred in 2 of 4 patients and 3 of 7 eyes. Median length of follow-up was 10 months (range, 7 to 21 months). No eyes required a third cryospray treatment in this case series. Average time to recurrence was 3.6 months in the 3 eyes re-treated with cryotherapy. Patient 2 is shown in Figure 13 before cryotherapy and 1 year after cryotherapy for SLK. Vision was the same in all patients before and after cryotherapy.

VERNAL KERATOCONJUNCTIVITIS

Cases 1 and 2 (Table 6) are from a 9-year-old white boy who presented to the Casey Eye Institute cornea clinic in January 2005 with severe photophobia, mucus discharge, bilateral eye pain, and itching. Clinical signs of VKC were evident, including giant papillae on the palpebral conjunctiva (giant papillary conjunctivitis) underneath both upper eyelids and corneal shield ulcers in both eyes (Figures 8 and 9). Topical treatment with artificial tears and ointment, topical olopatadine, topical cyclosporine, topical loteprednol, and topical NSAID eye drops resulted in minimal improvement. The loss of vision from the shield ulcers resulted in Snellen visual acuity of 20/70 in the right eye (OD) and 20/400 in the left (OS).

One month after initial presentation, liquid nitrogen cryotherapy was performed in the operating room with the patient under general anesthesia because of his young age (Figure 14). One month postoperatively, his vision had improved to 20/20 OD and 20/50 OS. The symptoms of itching, pain, and photophobia were absent, and the shield ulcers had healed with some residual scarring of the superficial stroma of the cornea OS. The giant papillae had flattened out, with a decrease in the number, size, and elevation of the remaining papillae. However, recurrence of giant papillae was already evident at 1 month (Figure 15).

TABLE 4. CASE REPORTS OF PATIENTS WITH ADVANCING WAVELIKE EPITHELIOPATHY TREATED WITH LIQUID NITROGEN CRYOTHERAPY

CASE	AGE	GENDER	EYE	VISION PRE-CRYO- THERAPY	VISION POST-CRYO- THERAPY	RESOLVED	POSSIBLE ETIOLOGY	OTHER EYE DISEASE	FOLLOW- UP DURATION (mo)
1	77	Female	Right	20/50	20/50	14 days	Glaucoma eye drops: dorzolamide hydrochloride–timolol maleate solution (Cosopt)	Dry eye, cataract, glaucoma	15
2	77	Female	Left	20/40	20/40+	14 days	Glaucoma eye drops: dorzolamide hydrochloride–timolol maleate solution (Cosopt)	Dry eye, cataract, glaucoma	16
3	83	Male	Left	20/60	20/50	14 days	Corneal transplant	Cataract, irregular astigmatism	18
4	76	Male	Left	20/40	20/40+	14 days	2 corneal transplants Glaucoma eye drops: latanoprost ophthalmic solution (Xalatan) and dorzolamide hydrochloride–timolol maleate solution (Cosopt)	Cataract, glaucoma, irregular astigmatism	12
5	78	Male	Left	20/40	20/40+	14 days	Glaucoma eye drops: dorzolamide hydrochloride–timolol maleate solution (Cosopt)	Cataract, glaucoma	13

TABLE 5. CASE REPORTS OF PATIENTS WITH SUPERIOR LIMBIC KERATOCONJUNCTIVITIS TREATED WITH LIQUID NITROGEN CRYOTHERAPY

PATIENT	AGE	GENDER	EYE(S) TREATED	RACE	THYROID DISEASE	RECURRENCE	FOLLOW-UP DURATION (mo)
1	78	Female	Both	White	Hyper-thyroidism	Yes OD (3 mo)	11
2	51	Female	Both	White	No	Yes OU (3 mo)	21
3	72	Female	Both	White	Hashimoto thyroiditis	No	9
4	56	Female	Right	Latino	No	No	7

Patients 1 and 2 had re-treatment within 2 weeks of recurrence. Disease resolved after subsequent liquid nitrogen cryospray.

In January 2006, 1 year later, the patient presented with recurrence of VKC. Cryotherapy was repeated, resulting in resolution of symptoms and signs of VKC within 1 month. During the summer months (June through August), the patient's symptoms and signs recurred but thus far have not required further intervention beyond topical treatment with loteprednol and lodoxamide.



FIGURE 13

Superior bulbar conjunctiva in a patient with superior limbic keratoconjunctivitis before and after cryotherapy (patient 2, Table 5).

TABLE 6. CASE REPORTS OF PATIENTS WITH VERNAL KERATOCONJUNCTIVITIS TREATED WITH LIQUID NITROGEN CRYOTHERAPY

CASE	AGE	EYE	GENDER	RACE	TREATMENT BEFORE CRYOTHERAPY	REPEAT CRYOTHERAPY	RECURRENCE	FOLLOW-UP DURATION (mo)
1*	9	Right	Male	White	Artificial tears, olopatadine, cyclosporine, loteprednol eye drops, topical NSAIDs, OD	Yes	Yes (1 yr)	24
2*	9	Left	Male	White	Artificial tears, olopatadine, cyclosporine, loteprednol eye drops, topical NSAIDs, OS	Yes	Yes (1 yr)	24
3	23	Left	Male	Latino	Olopatadine eye drops, Mucomyst 10% eye drops, loteprednol eye drops	No	Yes (9 mo)	18

NSAIDs, Nonsteroidal anti-inflammatory drugs (eye drops).

*Cases 1 and 2 are the same patient.

Case 3 (Table 6) is the report of a 23-year-old Latino man who was diagnosed with severe VKC in May 2003. He presented with red irritated eyes and severe photophobia with mucus discharge. On examination, he had corneal filaments and a severe giant papillary conjunctivitis involving the palpebral conjunctiva of both upper eyelids (Figure 16). He was treated initially with topical olopatadine eye drops, 10% Mucomyst to dissolve the filaments, and topical loteprednol 4 times daily. On subsequent follow-up visits, over the course of 2 years, his symptoms worsened.



FIGURE 14

Liquid nitrogen cryoprobe applied to the palpebral conjunctiva of a patient treated for vernal keratoconjunctivitis (case 1, Table 6).



FIGURE 15

Some flattening and resolution of the giant papillae in a patient with vernal keratoconjunctivitis 1 month after treatment with excision and cryotherapy. Same patient as shown in Figure 14 (case 1, Table 6).

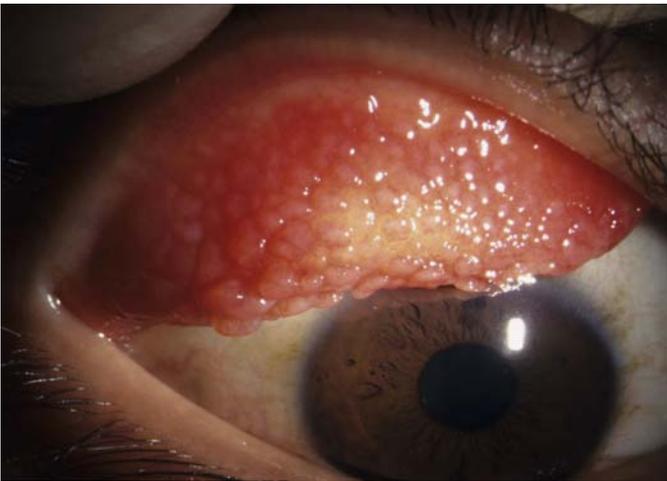


FIGURE 16

A patient with giant papillary conjunctivitis in vernal keratoconjunctivitis (case 3, Table 6).

In April 2005 this patient underwent liquid nitrogen cryotherapy, performed with a Brymill E tip (0.0130-inch aperture) cryospray to the palpebral conjunctiva of his right upper eyelid using a double freeze-thaw technique. His symptoms improved immediately, and he was doing well 3 months postoperatively; however, at 9 months after cryotherapy, symptoms and signs of VKC had returned. Cryotherapy was not repeated. He continues management with topical loteprednol and olopatadine eye drops.

The median length of follow-up for both patients (3 eyes) was 24 months, and the median time to recurrence was 12 ± 1.7 months.

DISCUSSION

From the evidence presented in these case series, liquid nitrogen cryotherapy to the ocular surface is effective in treating conjunctival amyloidosis, primary pterygia, AWLE, and SLK. It is less effective in the long term for treating recurrent pterygia and palpebral VKC. In this study, recurrent pterygia and VKC did improve initially; however, usually by 1 year after treatment, the diseases had recurred. Four of 6 patients presenting to Casey Eye Institute with recurrent (previously undergoing surgery) pterygia had another recurrence after cryotherapy. This recurrence occurred in just over 3 months postoperatively in 3 of the 4 Latino patients. This may indicate that cryotherapy in this subset of patients is contraindicated. The VKC recurred in approximately 1 year in the small subset of 3 eyes treated with liquid nitrogen cryotherapy.

Conjunctival amyloidosis, AWLE, and SLK are relatively rare conditions not commonly encountered in clinical practice. Severe VKC, which requires surgery because of vision-threatening shield ulcers or giant papillae, is rare. Also, pterygia requiring surgery are more common in states and countries closer to the equator; a clinical practice in Oregon probably will not treat as many patients with pterygia as in regions closer to the equator. Because of this, there are relatively few patients in each case series, which makes it difficult to generalize the results to a much larger patient population. In addition, it is unknown if the effects will be permanent for the eye diseases that benefited from therapy (ie, conjunctival amyloidosis, primary pterygia, SLK, and AWLE). Alternatively, the benefits of cryotherapy could last longer in the recurrent pterygia subset or the VKC group if a larger patient population was available.

Nonetheless, there is evidence that liquid nitrogen cryotherapy can effectively treat the conditions presented, with long-lasting effect in primary pterygia, conjunctival amyloidosis, SLK, and AWLE. The reason cryotherapy may work for some surface eye diseases has to do with the etiology of the underlying ocular disease and, perhaps, the way cryotherapy is applied. For example, a cryospray was used in treating AWLE and SLK, while for pterygia, amyloidosis, and VKC, a cryoprobe was used to directly contact the surface of the globe or the palpebral conjunctiva. In addition, excision prior to cryotherapy was performed in some conjunctival amyloid and VKC cases and all cases of pterygium. No excision was performed prior to cryotherapy in cases of SLK and AWLE. It is important to describe the etiology and possible mechanism of the benefits of cryotherapy for each ocular surface disease presented.

CONJUNCTIVAL AMYLOIDOSIS

Conjunctival amyloidosis is uncommon and rarely associated with systemic disease.^{88,89} The etiology of primary ocular localized conjunctival amyloidosis is not fully elucidated; however, many researchers feel there is an immunoglobulin light-chain deposition in the conjunctiva. The exact reason for such protein collections is unknown, but they could be associated with a benign localized B-lymphocyte lineage disorder that produces free immunoglobulin light chains that serve as precursors to amyloid deposits.

Liquid nitrogen cryotherapy may be therapeutic for conjunctival amyloidosis because it disrupts blood supply to the amyloid deposits. Liquid nitrogen cryotherapy damages small-caliber blood vessels and creates a hemostasis effect within blood vessels, which leads to cellular ischemia in the surrounding tissue. The lack of blood supply to amyloid deposits may contribute to tumor regression. The surgical debulking prior to cryotherapy may be beneficial in allowing the freezing to reach the deeper blood supply.

In addition, it is known that liquid nitrogen cryotherapy is effective in treating conjunctival lymphoma.² Prior studies have demonstrated a benign local monoclonal B-cell deposition in conjunctival amyloidosis.⁹⁰⁻⁹² Conjunctival amyloidosis is a result of deposition of proteins from these lymphocytes, and the cryotherapy is probably effective at killing these lymphocytes. It is possible that the destruction of the benign B-cell clones will prevent deposition of amyloid.

There have been no significant advances in the treatment of localized conjunctival amyloidosis, and the mainstay of treatment is surgical debulking, with subsequent debulking surgery should the amyloidosis recur.^{88,90} Demirci and coworkers⁸⁹ reviewed this condition by analyzing clinical and pathologic reports from prior publications. Outcome data was available on 30 subjects who had surgery for conjunctival amyloidosis, with 8 subjects having recurrent and progressive conjunctival amyloidosis (27% recurrence). Two of 4 patients had recurrence of conjunctival amyloidosis in the cases presented here; however, subsequent cryotherapy has eradicated this condition in all patients to date. Liquid nitrogen cryotherapy, with or without surgical debulking prior to treatment, appears to be a safe and effective treatment.

PTERYGIA

These benign proliferations are thought to arise from activated and proliferating limbal epithelial stem cells. The pathogenesis of pterygia is unknown, but epidemiologic studies have implicated ultraviolet light, exposure to the environment, and chronic irritation as causative factors.⁹³⁻⁹⁵ These external factors may disrupt the signals to epithelial stem cells for apoptosis or proliferation.⁹⁶⁻⁹⁷ In essence, it is hypothesized that there may be a disruption in normal conjunctival cellular homeostasis.

In epithelial cells, normal tissue homeostasis is maintained by a tight coordination between cellular apoptosis and cellular proliferation.⁹⁸ From published reports, pterygium specimens display an abnormal number of apoptotic cells when compared with normal bulbar conjunctival tissue. There also is an abnormal expression of genes associated with the control of apoptosis, including *P53*, *BAX*, and *BCL2*.⁹⁹ Pterygia may arise as a result of incorrect control of cellular apoptosis rather than from an increase in proliferative capacity.⁹⁷

Liquid nitrogen cryotherapy after pterygium excision may work by direct cytotoxicity and by inducing apoptosis in proliferating epithelial stem cells. The cryogen may also be effective in eradicating the microvasculature of pterygia, as these tumors are highly vascularized. Prior research has shown liquid nitrogen cryotherapy to be effective in eradicating conjunctival vascular tumors.⁶⁶ Our case series of 18 patients (Table 2) had a 5.5% recurrence rate (Figure 11). This compares favorably with the recurrence rates of various adjunctive therapies, found in published reports to date.

For instance, Donaldson and Alfonso¹⁰⁰ report a 6% recurrence rate with conjunctival autograft, 13% with beta-irradiation, 29%

with mitomycin C, and 53% with excision alone after more than 5 years of follow-up. Küçükerdönmez and coworkers¹⁰¹ report a 10% recurrence with a conjunctival autograft and a 21% recurrence with an amniotic membrane graft after pterygium excision with over a year of follow-up. Luanratanakorn and associates¹⁰² reveal a 13% recurrence rate with conjunctival autograft and 28% with amniotic membrane transplantation after 6 months of follow-up. Mikaniki and Rasolinejad¹⁰³ compared mitomycin C 0.02% eye drop application for 4 days after pterygium excision to excision alone without adjunctive therapy in over 400 patients. At 1 year, there was a 20% recurrence rate for excision alone and 30% recurrence after 5 years. In the mitomycin-C treated group, there was a 1% recurrence rate at 1 year and 5 years. Bekibele and colleagues¹⁰⁴ studied 5-FU adjuvant treatment after excision compared to excision with conjunctival autograft with less than a year of follow-up. The recurrence rate was 11% with intraoperative 5-FU application and 12% with conjunctival autograft. Katircioğlu and coworkers¹⁰⁵ compared amniotic membrane graft to conjunctival autograft and autograft combined with mitomycin C with approximately 1 year of follow-up. The recurrence rate was 16% for amniotic membrane graft and 25% for autografts; for the combination of mitomycin C and autograft, there were no recurrences in 8 eyes.

In this case series, the absence of recurrence in the majority of patients with a median follow-up of 24.5 months is strong evidence that cryotherapy is an effective adjunctive therapy. Since most pterygia recur within the first year after treatment, absence of recurrence beyond 1 year may mean that cryotherapy can be curative for this surface eye disease.¹⁰⁶

In the recurrent pterygia treated with liquid nitrogen cryotherapy as adjunctive therapy after excision, the recurrence rate was 66%. Moreover, the recurrence occurred very rapidly in the Latino patients (Table 3). The recurrences may be related to the inadequacy of cryotherapy for recurrent pterygia and also possibly to surgeon technique in removal of the primary pterygium. Nevertheless, liquid nitrogen cryotherapy is not recommended for recurrent pterygium based on the small case series presented.

ADVANCING WAVELIKE EPITHELIOPATHY

The etiology of AWLE is probably multifactorial. It has been hypothesized that prior ocular surgery, contact lens wear, contact lens solution, glaucoma drop toxicity, and underlying inflammatory or dermatologic disorders may all cause this condition.¹⁰⁷

AWLE as a result of topical ocular medication toxicity is a distinct possibility for the subjects presented here, as the patients in cases 1, 2, 4, and 5 were using intraocular pressure-lowering eye drops and those in cases 3 and 4 used topical corticosteroids and antibiotics after corneal transplantation. It has been shown that long-term use of topical eye drops may result in an increase in inflammatory cells in the conjunctiva and in corneal keratinization, scarring, and neovascularization.^{108,109}

Confocal microscopy reveals the presence of atypical elongated cells oriented centripetally with hyperreflective nuclei.¹¹⁰ Pathologic diagnosis is consistent with unremarkable corneal epithelium when stained with hematoxylin-eosin, with no evidence of cytologic alterations or dysplastic change. Full-thickness conjunctival biopsies have revealed parakeratosis of the conjunctival epithelium with underlying focal mononuclear cell infiltrates compressing and extending into the overlying epithelium.¹⁰⁷ The only published treatment method to date describes the application of 1% silver nitrate solution to the corneoscleral limbus with removal of the corneal epithelium through debridement.¹⁰⁷

It is possible that liquid nitrogen therapy works by killing the abnormal, benign surface epithelial cells that are growing from the corneoscleral limbus onto the surface of the cornea. Because the liquid nitrogen spray creates a superficial freeze when applied for less than 1 second, the unwanted corneal epithelium can be frozen without damage to the underlying corneal stroma or endothelium. The cytotoxic effects of freezing appear to obliterate the AWLE within 2 weeks with long-term results (Table 4).

In this case series, there have been no recurrences in any of the patients after more than a year of follow-up. This compares favorably to the reported recurrence rate of 5 cases in 11 eyes (45%) in the only other case series describing treatment of AWLE.¹⁰⁷

Advancing wavelike epitheliopathy is a relatively rare condition that may go unrecognized by many clinicians, as it is associated with common conditions such as chronic topical ocular therapy, prior ocular surgery, and contact lens wear. Liquid nitrogen cryotherapy, using the spray technique described, is a safe and effective means of eradicating AWLE and can be used as an alternative to silver nitrate 1% solution.

SUPERIOR LIMBIC KERATOCONJUNCTIVITIS

The etiology of SLK is a subject of some debate; however, many researchers believe there is a mechanism leading to soft tissue microtrauma between the superior palpebral and superior bulbar conjunctival surfaces from normal repetitive eye blinking in susceptible individuals.¹¹¹⁻¹¹³ Others have postulated an insufficient local tear supply.¹¹⁴⁻¹¹⁶ Regardless of etiology, a multitude of treatments are suggested for SLK, including thermocautery, chemocautery, conjunctival resection, punctal occlusion, topical application of autologous serum, topical cyclosporin A, topical ketotifen fumarate, bandage contact lenses, topical iodoxamide tromethamine, botulinum toxin, and topical vitamin A eye drops.¹¹⁴⁻¹²¹ The fact that there are so many treatments frequently means that no single treatment is adequate, and that the disease is a result of a combination of factors, including dry eyes, mechanical trauma, local inflammation, and the effect of Graves disease on the eyes.¹²²

The microtrauma hypothesis provides a likely explanation for SLK and also for the effectiveness of cryotherapy in the case series presented here (Table 5). One of the mainstays of treatment for this disease is chemocautery, usually with a silver nitrate solution or silver nitrate stick applied to the superior conjunctiva.¹²³⁻¹²⁶ Another common treatment is conjunctival resection.^{112,127,128} Both of these treatments essentially remove the theoretically redundant superior conjunctiva from the surface of the globe. Therefore, the chronic microtrauma from repetitive blinking does not occur.

Liquid nitrogen cryotherapy acts in the same way; it removes the redundant superior conjunctiva by causing a scar similar to that produced by chemocautery to form between the superior bulbar conjunctiva and the underlying Tenon capsule and sclera.

In the 7 eyes of 4 patients in this series (Table 5), the effects of liquid nitrogen cryospray appeared to last for months without adverse ocular side effects. In 3 of 7 eyes, disease recurred after a single cryotherapy treatment, but there were no recurrences after subsequent cryotherapy. The published literature has a paucity of reports on recurrence of SLK, and the recurrence varies depending on the type of treatment. Passons and Wood¹²⁷ performed conjunctival resection for SLK and had recurrence in 2 of 10 patients. Ohashi and colleagues¹²¹ treated SLK with vitamin A eye drops with benefit in 10 of 12 patients. Udell and coworkers¹²⁴ used thermocautery in the treatment of SLK, with recurrent disease in 5 of 11 patients (45%). It is hoped that the effects of cryotherapy will be permanent, and if the mechanical theory of microtrauma is correct, then the scarring formed from cryospray to the surface of the globe should last permanently.

VERNAL KERATOCONJUNCTIVITIS

The giant papillary changes in VKC are collections of neutrophils, eosinophils, lymphocytes, and other leukocytes surrounding a central vascular core.¹²⁹ It was hypothesized that the giant papillae were susceptible to cryotherapy, as the vascular endothelium of the central vascular core is destroyed with liquid nitrogen freezing. The VKC recurred in 1 year or less in all 3 cases after cryotherapy (Table 6). However, the resolution of symptoms and restoration of vision were rapid after treatment, as the shield ulcer in patient 1 resolved within 1 month. In addition, in the short term, the giant papillae of the VKC flattened out with a decrease in number, size, and elevation by 1 month after treatment.

Published reports on cryotherapy for VKC reveal mixed results. Sankarkumar and coworkers¹³⁰ studied 30 eyes of 15 patients with VKC, treated with the cryogen carbon dioxide. Reported recurrence was only 3.3% at 1 year; patients also received oral aspirin. Mtanda and Sangawe¹³¹ studied 34 eyes with VKC, treating the condition with a carbon dioxide cryogen. Disease recurred in 2 eyes (6%) at 5 months. However, Singh¹³² reported a 22% recurrence rate with short-term follow-up using a carbon dioxide cryogen, and Abiose and Merz,¹³³ also using a carbon dioxide cryogen, had a 50% recurrence of giant papillae at just 4 weeks posttreatment. All authors of these studies did report that patients had immediate improvement in symptoms, much like what was observed in the 3 cases reported here.

Still, because the VKC recurred and the patients' eye conditions are essentially the same 1 year after surgery in this study, liquid nitrogen cryotherapy should probably be avoided. The freezing may kill the central vascular core early on; however, the papillae reappear and the central vascular core rejuvenates in a relatively short amount of time after cryoinjury. Prior research has shown that large-caliber blood vessels are resistant to cryoinjury, and the central vessels of the VKC papillae are probably too large to damage permanently.⁶⁹ The cryotherapy may also damage the healthy palpebral conjunctiva, leading to scarring and dry eye, and thus has the potential to increase patient morbidity in the long term. From the results of the small case series presented, liquid nitrogen cryotherapy for VKC is not recommended.

CONCLUSION

The case reports presented here are the first describing the method and results of liquid nitrogen cryotherapy for conjunctival amyloidosis, primary pterygia, recurrent pterygia, AWLE, SLK, and VKC. Frequently, an excisional biopsy or debulking surgery may need to be performed prior to cryotherapy with either a cryospray or cryoprobe. The choice depends on the surgeon and the disease, and there is room for variation in the method chosen.

Published reports describe multiple treatment options for SLK, pterygia, and VKC, indicating that no single treatment is efficacious enough to be the answer. Conjunctival amyloidosis and AWLE are rare, and the only published treatments are surgical debulking for the former and silver nitrate solution with debridement for the latter. To evaluate a new approach, liquid nitrogen cryotherapy was tried as a treatment for these surface eye diseases.

Liquid nitrogen cryotherapy is recommended as an adjunctive therapy after pterygium excision. It is also beneficial in the treatment of conjunctival amyloidosis; however, if significant amyloid is present, a debulking surgery should be performed prior to cryotherapy. Superior limbic keratoconjunctivitis can be treated effectively with direct liquid nitrogen cryospray, as can AWLE. At this time, liquid nitrogen is not recommended in the treatment of VKC and recurrent pterygia (Table 7).

TABLE 7. SUMMARY OF LIQUID NITROGEN CRYOTHERAPY FOR SURFACE EYE DISEASE

EYE DISEASE	ADVANTAGE OF CRYOTHERAPY	DISADVANTAGE OF CRYOTHERAPY	ALTERNATIVE TREATMENTS
Conjunctival amyloidosis	Improves condition after excisional biopsy. Relatively long-term resolution of disease. Repeat excision and cryotherapy effective with recurrent amyloidosis.	Recurrence in half of cases presented an average of 1 year after excision and cryotherapy. Subsequent excision and cryotherapy may be needed.	Excisional biopsy and surgical debulking. ^{89,91}

TABLE 7(CONTINUED). SUMMARY OF LIQUID NITROGEN CRYOTHERAPY FOR SURFACE EYE DISEASE

EYE DISEASE	ADVANTAGE OF CRYOTHERAPY	DISADVANTAGE OF CRYOTHERAPY	ALTERNATIVE TREATMENTS
Primary pterygium	Excision and cryotherapy safe and effective in this case series with a 5.5% recurrence rate and more than 2 years of follow-up.	No adverse ocular events observed in this case series after excision and cryotherapy.	Conjunctival autograft ¹⁰⁵ Amniotic membrane graft ¹⁰² Mitomycin C ¹³⁴ 5-FU ¹⁰⁴ Beta-irradiation ¹⁰⁰ Excisional biopsy alone ¹⁰³
Recurrent pterygium	Short-term resolution of recurrent pterygia observed.	Four of 6 pterygia recurred again, with 3 of 4 recurrences at approximately 3 months after excision and cryotherapy.	Same as for primary pterygium.
AWLE	Long-term resolution with more than a year of follow-up in all 5 eyes treated with liquid nitrogen cryospray.	No adverse ocular events observed.	Silver nitrate solution ¹⁰⁸
SLK	All cases of SLK resolved with liquid nitrogen cryospray. Average follow-up approximately 1 year.	Two of 4 patients had recurrence of SLK within 3 months, which required subsequent cryotherapy with liquid nitrogen cryospray.	Silver nitrate solution ¹²⁴ Conjunctival resection ¹²⁸ Topical eye drops: autologous serum ¹¹⁵ cyclosporin A ¹¹⁶ ketotifen fumarate ¹¹⁷ bandage contact lens ¹¹⁸ lodoxamide tromethamine ¹¹⁹ Botulinum toxin ¹²⁰ Vitamin A ¹²¹
VKC	Short-term benefit realized in all 3 eyes treated.	Long-term cure did not occur in any eyes treated with liquid nitrogen cryotherapy with cryoprobe application to palpebral conjunctiva. Both patients had disease recurrence in less than 1 year on average.	Topical eye drops: steroids ¹²⁹ nonsteroidals ¹²⁹ ketotifen fumarate ¹³⁵ olopatadine HCl ¹³⁵ cyclosporine ¹³⁶ Contact lens ¹³⁷ Steroid injection ¹³⁸ Surgical resection ¹³⁹

Liquid nitrogen cryotherapy on the surface of the eye has proven effective in treating malignant and premalignant ocular surface tumors. In malignancies, a rapid freeze and slow thaw is the most tumoricidal, and this is effectively accomplished with liquid nitrogen, as it has the lowest boiling point and can provide the quickest freeze of the cryotherapy agents in use. None of the conditions described here are malignant, and it is possible they could be treated with alternative cryogens. Future research could address whether cryogens with higher boiling points, such as nitrous oxide, solid carbon dioxide, or freon could be effective in the treatment of the surface eye diseases described.

In addition, it is important to note that the techniques described here are typically not taught during ophthalmology residency training programs or in most fellowship programs. The research on the benefits of liquid nitrogen treatment of surface eye disease may progress as more practitioners learn these techniques.

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