CORNEAL ULCERS
Diagnosis and Management

Namrata Sharma
Rasik B Vajpayee

Forewords
Hugh R Taylor
Peter R Laibson

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Diagnosis and Management

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CORNEAL ULCERS
Diagnosis and Management

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Forewords
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Corneal Ulcers: Diagnosis and Management

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Dedicated to

My parents Dr Ramesh C Sharma and Maitreyi Pushpa, husband Subhash Chandra and daughter Vasavdatta

— Namrata Sharma

My wife Madhu and children Mihika and Shubhankar

— Rasik B Vajpayee
Corneal opacity and scarring is one of the leading causes of vision loss and blindness worldwide. Although cataract may be the leading cause of bilateral blindness, corneal scarring accounts for a significant portion of unilateral and bilateral vision loss. Corneal scarring may result from specific conditions such as trachoma, xerophthalmia or onchocerciasis, or from less specific causes such as microbial keratitis following often relatively trivial trauma. In some areas one quarter of blindness may be due to corneal scarring.

One hundred years ago, or even 50 years ago, our ability to treat microbial keratitis was extremely limited. The development of a broad range of antimicrobial agents, anti-inflammatory drugs, and vastly improved and faster diagnostic methods has revolutionized our ability to treat corneal ulcers. The successful outcome for the management of corneal ulceration depends on the prompt use of the appropriate antimicrobials and the careful management of the healing phase. This is easy to say, but is much more involved to achieve. It requires good clinical and diagnostic skills and excellent laboratory services to make the correct diagnosis so as to be able to select the appropriate antimicrobial. The careful management requires the early recognition and correct management of a host of possible complications.

Professor Rasik Vajpayee is a corneal surgeon of international renown. He was the Head of the very busy Cornea and Refractive Surgery Services at the RP Centre for Ophthalmic Sciences at the All India Institute of Medical Sciences and recently has taken over as Head of Cornea and Cataract Surgery at Centre for Eye research Australia, University of Melbourne. Both he and Dr Namrata Sharma have a profound knowledge and broad experience in the management of the whole range of corneal diseases and especially corneal ulceration. They have crystallized their experience into a beautiful set of clear and succinct guidelines.

This book builds on a systemic approach with a clear statement of the fundamental issues relating to corneal ulceration and the details that are important in the initial assessment. It covers in detail the microbiologic laboratory assessment and treatment options. The section on the surgical management is superb and sets out in a series of simple steps the way to successfully manage the various complications.

Professor Vajpayee and Dr Sharma have done us all a real service in compiling so much insight and experience into this easy to follow text. I highly recommend this book to all who have to manage patients with corneal ulceration.

Professor Hugh R Taylor
AC MD BS FRANZCO FRACS FAAO FACS
Ringland Anderson, Professor of Ophthalmology and Head
University of Melbourne, Department of Ophthalmology
Managing Director, Centre for Eye Research, Australia
Corneal Ulcers: Diagnosis and Management is a must-read reference and resource for every ophthalmologist interested in anterior segment ocular pathology. It contains the most up-to-date information about the recognition and treatment of this very severe and potentially blinding condition.

Dr. Rasik Vajpayee and Dr. Namrata Sharma have both published hundreds of papers and chapters in this area and have a vast experience in diagnosing and treating corneal ulcers. They have put their combined knowledge to excellent use by writing an outstanding textbook and guide to the management of these ulcers.

From the beginning chapters on the pathogenesis, microbiology and pharmacology of corneal ulcers, the writing is clear, concise and readily absorbed. It is not encyclopedic, but very practical, with superb color photographs and easily read box inserts highlighting the most significant material in the chapters.

Their chapter in Section 2 on the work up of a corneal ulcer is particularly illuminating, especially the many color illustrations of microbiological organisms and the havoc they can bring to the cornea. There is an excellent step by step approach to diagnosing and managing corneal ulcers, from the simple ones to the most complex.

Drs. Vajpayee and Sharma are particularly gifted in the field of microbiology, and their chapter on investigations of corneal ulcers in Section 2 is extremely well designed, with inclusive but not overwhelming tables on how to proceed with an ulcer work up. In Section 4, the chapters highlight specific types of microbial and immunologic keratitis, including pediatric and peripheral ulcerative keratitis. The writing throughout is again very clear and the photographs complement the text beautifully.

The surgical management section includes very high-quality illustrations on the use of glue and bandage contact lenses, conjunctival flaps, therapeutic keratoplasty and phototherapeutic keratectomy, with which both authors have extensive expertise, due to the severe and late-stage ulcerations treated in India.

I can strongly suggest, if not emphatically state, that this treatise will be a best-seller around the world and an invaluable aid in addressing the problems of corneal ulceration.

Peter R. Laibson  MD  
Professor of Ophthalmology  
Thomas Jefferson University School of Medicine  
Director Emeritus  
Cornea Department  
Wills Eye Institute  
Philadelphia, Pennsylvania
Corneal ulcer is a major cause of blindness in the developing world. The condition requires early recognition and prompt management to minimize the impact of disease process. There are many books available on the corneal and external diseases and include details on various aspects of corneal ulcer. However, most of these books carry enormous amount of information, some of which may not be required for the routine management of a case of infectious keratitis. We felt that there is a need for a book on the specific aspect of corneal ulceration that carries relevant, specific and practical information and can help general ophthalmologists in treating cases of corneal ulceration effectively. Our book includes a chapter highlighting a practical approach on how to examine a case of infectious keratitis and chapters on various types of keratitis. It also includes chapters on basic sciences relevant to corneal ulcer and provides comprehensive information on various management issues including surgical options, if required. We have tried to provide a precise format for our book and have written it in a user-friendly style. We hope that this book will serve as a useful guide for the residents as well as the general ophthalmologists.

Namrata Sharma
Rasik B Vajpayee
Acknowledgements

We would like to acknowledge Dr Tushar Agarwal, Dr M Vanathi, Dr Tishu Saxena and Dr Gunjan Prakash for their useful inputs. We would also like to thank Departments of Microbiology of All India Institute of Medical Sciences and St. Vincent Hospital for the photographs. Our heartfelt gratitude to Ms Meena Verma, Ms Sudha, and Ms Lata for helping us with clinical photography.
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SECTION 1
Cornea is the principal refractive surface of the human eye and along with sclera forms the outermost coat of the eyeball. It constitutes up to one-sixth of the entire eyeball. The corneal epithelium is derived from the surface ectoderm and the mesoderm gives rise to Bowman’s layer, stroma, Descemet’s membrane and endothelium. The average diameters of the cornea vary from 11 to 12 mm horizontally and 9 to 11 mm vertically. Cornea accounts for approximately 48 diopters of the power. The posterior surface of the cornea is more spherical than the anterior surface and the central cornea is thinner (520 μm) than the peripheral cornea (650 μm or more). The tear film covers the anterior corneal surface and the posterior corneal surface is in contact with the aqueous.

**PRE-CORNEAL TEAR FILM**

The tear film forms an important defense mechanism against the microbial infection. It is 7 μm thick and has a volume of 6.5 ± 0.3 μL. The tear film is made up of an outer lipid layer (0.1 μm), middle aqueous layer (7 μm) and innermost mucin layer (0.02 to 0.05 μm). It is now believed that the tear film is actually a two-layered structure where under the lipid layer lies an aqueous–mucin gel, in which the mucins have a decreasing gradient of concentration from the epithelium to the surface. Tear film keeps the corneal surface moist and prevents the adherence of microbes. A deficient pre-corneal tear film may predispose to the occurrence of corneal infection.

More than 98 percent of the volume of the tears is water. The tear film has many essential substances such as electrolytes, glucose, immunoglobulins, lactoferrin, lysozyme, albumin and oxygen. It also has many biologically active substances such as histamines, interleukins, prostaglandins and growth factors. Some of these factors modulate corneal epithelial migration, proliferation and differentiation.

**STRUCTURE OF CORNEA**

Cornea essentially consists of 5 layers namely—epithelium, Bowman’s layer, stroma, Descemet’s membrane and the endothelium (Figs 1.1 to 1.3).
EPITHELIUM

Corneal epithelium has a thickness of 50-90 μm and is comprised of five to seven layers of stratified, squamous and non-keratinized cells (Fig. 1.2). It represents 10 percent of the total corneal thickness.

The cells of corneal epithelium can be classified into three categories—the superficial squamous cells, middle wing cells and deeper basal cells.

Superficial Squamous Cells

Superficial or squamous cells form the outermost 1-2 layers of corneal epithelial cells. They are the oldest epithelial cells and they disintegrate and shed into the tear film by the process of desquamation. These cells have microscopic projections (microvilli, reticulations, microplicae) and fibrillar glycocalyx on their which interacts with the mucinous tear film. The epithelium turns over approximately every 7 to 14 days.

The superficial cells are connected to each other by desmosomes and junctional complexes. This complex consists of tight junctions, which surround the entire cell, and resist the flow of fluid through the epithelial surface.

Middle Wing Cells

The middle layer of the corneal epithelium consists of the wing cells, which have lateral, thin wing like extensions emanating from a more rounded cell body. The adjacent cells are joined by desmosomal junctions and gap junctions.

Deep Basal Cells

The basal cells are cuboidal to columnar in shape and are 8 to 10 μm in diameter. Posteriorly, the cells are flat and are supported by a basal lamina to which they are attached by hemi-desmosomes. The basal cells are metabolically active and divide giving rise to the wing and the superficial cells.

While corneal epithelium acts as a tough protective shield against microorganisms and foreign bodies, it has some permeability to small molecules including glucose, sodium oxygen and carbon dioxide.

BASEMENT MEMBRANE

The basal cells of corneal epithelium are attached by hemi-desmosomes to a basement membrane, which is located between the corneal epithelium and the Bowman’s membrane and contains type IV and type VII collagen and glycoproteins. The basement layer of corneal epithelium has two parts: Lamina lucida (superficial) and Lamina densa (deep).

BOWMAN’S LAYER

The Bowman’s layer is an acellular membrane like zone, 8 to 14 μm thick and has numerous pores for the passage of corneal nerves into the epithelium. Ultrastructurally it is made up of a fine meshwork of uniform collagen fibrils of type I and III.

CORNEAL STROMA

The corneal stroma is approximately 500 μm thick and comprises 90 percent thickness of the cornea and is located between the Bowman’s layer and Descemet’s membrane (Fig. 1.2). It is composed of lamellae formed from flattened bundles of collagen, stromal keratocytes and ground substances like keratan sulphate. Collagen (type I is the major constituent, others are III and VI) is the major structural component of corneal stroma. There are 200 to 250 bundles of collagen fibrils. Each bundle extends the width of the cornea and is 2 nm thick and 9 to 260 nm wide. The collagen fibers are arranged in a regular manner, parallel to the corneal surface. Such arrangement and equal spacing of collagen fibers creates a lattice or three-dimensional diffraction grating, which is responsible for the ability of the cornea to scatter 98 percent of the incoming light rays. The lamellae in the posterior part of the stroma have an orthogonal layering, i.e. the bundles are at right angles to each other.
In the anterior one-third of the stroma, the lamellae have a more oblique layering.

The primary glycosaminoglycans of the stroma are keratin sulfate and chondroitin sulfate, which occur in the ratio 3:1. The lamellar stroma is secreted and maintained by stromal fibroblasts called the keratocytes, which occupy 3-5 percent of the stromal volume. They are responsible for the maintenance of stromal components and they synthesize collagen degradative enzymes such as matrix metalloproteases (MMPs). The MMPs are particularly important in the pathogenesis of peripheral ulcerative keratitis as they accumulate in the tears and trigger an autoimmune response involving the ocular tissue. Keratocytes undergo cellular differentiation in response to injury converting into fibroblasts. Keratocytes usually lie between the lamellae being flat with long attenuated processes extending from a central cell body in all directions. Depletion of keratocytes is a characteristic feature of Acanthamoeba keratitis.

**DESCEMET’S MEMBRANE**

Descemet’s membrane is the basement membrane of the corneal endothelium and is synthesized by the endothelium. At birth, the human Descemet’s membrane is 3 μm wide but in adulthood, the width increases to 12 μm (Fig. 1.3). There are two distinct regions—antero, one-half to one-third, which is banded, and posterior two-third, which is non-banded.

In certain types of bacterial keratitis and Mooren’s ulceration, this membrane remains intact and protrudes as a descemetocele due to the intraocular pressure following dissolution of overlying stroma.

**ENDOTHELIUM**

The corneal endothelium is a single layered, low cuboidal endothelium. There are approximately 400,000 cells, 4 to 6 μm thick. These cells are hexagonal in shape and 20 μm wide. The endothelial cells have tight lateral interdigitations, preventing seepage of aqueous humor into the stroma. Further, specific functional complexes are also present near the apical membranes.

The number of endothelial cells, present decreases with age at the rate of 0.3 to 0.6 percent per year. At birth, the cell densities range from 3,500 to 4,000 cells/mm² whereas an adult has densities of 1400 to 2500 cells/mm². As cells decrease in number, they become thinner and attenuated. Cornea loses it clarity when the endothelial cell densities reach 400-700 cells/mm² below which corneal edema occurs.

Unlike corneal epithelium, endothelial cells cannot undergo mitosis after birth. The endothelial cells are linked to each other by junctional complex structures and presence of gap junctions but no desmosomes are present.

The endothelial cells do not replicate in human beings. The endothelial cells decrease in density with increasing age, raised intraocular pressure, following intraocular surgery and inflammation.

The corneal endothelium plays a major role in maintaining stromal hydration (which is normally 78%) through the Na-K-activated adenosine triphosphatase (ATPase) present in the basolateral borders of the cells.

**INNERVATION OF THE CORNEA**

The cornea is primarily supplied by the sensory nerves derived from the ciliary nerves of the ophthalmic branch of the trigeminal nerve. The long ciliary nerves supply the perlimbal nerve ring. Nerve fibers penetrate the cornea in the deep peripheral stroma radially and then course anteriorly forming a terminal subepithelial plexus. The nerve fibers lose their myelination soon after penetrating the clear cornea and enter the Bowman’s layer and terminate at the level of the wing cells. An autonomic sympathetic supply is also present in the cornea.

The physiologic role of corneal innervation is unclear. Presence of corneal sensation is vital to the maintenance of the integrity of the cornea. In cases of herpes simplex, herpes zoster and diabetes, corneal sensations are diminished and this may lead to persistent epithelial defects or delayed epithelial wound healing.

**BLOOD SUPPLY**

The cornea is one of the few avascular tissues in the body. The normal healthy cornea does not have any blood vessels. The anterior ciliary artery derived from the ophthalmic artery forms an arcade at the limbus.

**OXYGEN AND NUTRITIONAL SUPPLY**

Oxygen is supplied primarily from the diffusion from the tear film. Oxygen from the air is also dissolved in the tear fluid. To a lesser extent oxygen is also obtained
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from the aqueous and the limbal vessels. Thus during contact lens wear the oxygen diffusion from the atmosphere is less. Also, in patients who sleep with their contact lenses on, the metabolism converts from aerobic to anaerobic causing lactic acid to accumulate in the cornea.

Applied Physiology of Cornea

The basis of corneal physiology consists of the understanding of corneal epithelial barrier, endothelial barrier and metabolic pump functions. The factors which affect the hydration of the cornea include the following: The corneal epithelial barrier, the endothelial barrier, the metabolic pump function, evaporation and the intraocular pressure. If either of these barriers is compromised, it manifests as corneal swelling. A greater increase in the corneal thickness occurs when endothelial cells are compromised (which may be as much as more than two times) as compared to the damage of the epithelial cells. Metabolic pump function also plays a vital role as the corneal stroma swells up due to increased tonicity of the stromal components, which contains collagen, salts and proteoglycans.

The corneal stromal swelling pressure is 60 mm Hg and corneal stromal swelling occurs if endothelial barrier is disrupted as the intraocular pressure of 15 mmHg is unopposed and the aqueous seeps into the stroma.

Both the anterior and the posterior surfaces of the cornea contribute to its optical function. The total refractive index of the cornea is the sum of the refraction at the two interfaces as well as the transmission properties of the tissue. The refractive index of the air, tear, cornea and aqueous humor are 1.0, 1.336, 1.376 and 1.336 respectively. The refractive power at the curved surface is determined by the refractive index and the radius of curvature. Refractive power at the central cornea is about +43 diopters and is the sum of the refractive power at the air-tear (+44 diopeters), tear-cornea (+5 diopeters) and cornea-aqueous humor (+6 diopeters) interfaces.

The anterior and posterior corneal stroma is different morphologically. Dermatan sulphate, which has greater water retentive property, is located more in anterior stromal layers whereas keratin sulphate is located more in the posterior stromal layers. Hence, clinically, if edema is restricted to the posterior layers, it resolves more easily.

CORNEAL TRANSPARENCY

The corneal transparency is unique and is attributed to its avascular status, peculiar arrangement of collagen fibers, absence of myelin sheath in its nerves and corneal endothelial pump.

According to the lattice theory proposed by Maurice, the cornea maintains its transparency because the collagen fibrils are of equal diameter (275-350Å) and are equidistant from each other. Thus the incident ray scattered by each collagen fiber is cancelled by the interference of other scattered ray which allows it to pass through the cornea. Corneal decompensation due to corneal hydration occurs as the proteoglycans within the corneal lamellae imbibe water and this equilibrium is disturbed leading to a loss of transparency.

The biochemical and physical properties of the stroma are normally maintained by the presence of a functional epithelial and endothelial barrier and a metabolic pump function so that the water content is maintained at 78 percent.

Normal Defense Mechanism

Cornea along with conjunctiva and tear film act as a major component of ocular defense system against the microbial infections. While corneal epithelium acts as a mechanical barrier, the cellular and chemical components of conjunctiva and pre-corneal tear film act as biologic protective systems.

There are multiple barriers to ocular infection. Anatomically, the eye is protected from the introduction of microbes due to trauma by the surrounding bony structure of the protruding orbital rim. The cilia protect the eyelid by a rapid blink reflex.

The eyelid skin, cilia and adnexal surfaces are normally inhabited by nonpathogenic/saprophytic aerobic and anaerobic bacteria which decreases the chances of colonization by the pathogenic microbes. Additionally, the intact epithelial surfaces of the conjunctiva and cornea provide a formidable barrier to the invasion by the microorganisms.

The presence of an intact tear film and its drainage by the lacrimal apparatus acts as an intrinsic barrier to infection. The microorganisms, foreign bodies and desquamated epithelial cells are continuously washed out of the eye due to blinking and lacrimal drainage system. The mucus layer of the tear film also provides antimicrobial properties which inhibits the bacterial adhesion to the epithelial cell layer. Other molecules
which provide antimicrobial protection are microbe-specific antibodies (especially IgA) and non-specific antimicrobial molecules such as complement, lactoferrin, lysozyme and β-lysin (Table 1.1).

The conjunctiva has conjunctiva associated lymphoid tissue (CALT) wherein, immunity is initiated by exposure to exogenous antigen by the production of antibody which is IgA isotype.

**References**

Introduction

Corneal ulceration occurs due to the host cellular and immunologic responses to the offending agent which may be bacterial, viral, fungal or protozoal organism. Sometimes it is sterile corneal ulceration, which may occur due to systemic dermatologic or connective tissue disease and chemical or thermal injuries.

The host cellular responses are mainly responsible for corneal destruction in infections and sterile corneal melting. In all cases, stromal melting is preceded by a corneal epithelial defect. The ulceration occurs secondary to the action of tissue collagenases. The polymorphonuclear cells (PMNs) are secreted in response to the corneal insult, which secrete various lytic enzymes such as collagenase, elastase and cathepsin causing destruction of the cornea. Simultaneously, reactive fibroblasts, synthesize collagen and cause repair of the cornea.

Apart from the infective processes, the immunologic mechanisms consequent to infection may also play a role. For example, in herpes simplex interstitial keratitis stromal cellular destruction occurs due to immunologic mechanisms as a consequence of acquisition of herpes antigens, thereby resulting in an influx of PMNs and phagocytes, which cause tissue destruction.

For the reparative phase of corneal ulcer, the interaction between the keratocytes and blood vessels is essential. Stromal vascularization inhibits the ulcerative process as nutrients (such as ascorbate) and antiproteases are delivered by the vessels to the ulcerated area.

STAGES OF CORNEAL ULCER

The course of events, which occur in the process of corneal ulceration, can be divided into three stages.

Stage 1: Progressive Stage

In the progressive stage, the ulcer is usually saucer shaped and is associated with gray zone of infiltration (Fig. 2.1). In this stage, the microbes adhere to the epithelium, release toxins and enzymes and cause tissue destruction (Fig. 2.2).
Adhesion of the organisms is facilitated by bacterial pili and a glycocalyx envelope in bacteria such as Pseudomonas and Gonococcus. In response to this, PMNs are generated at the ulceration site. The PMNs originate from the tears initially and limbal vessels consequently in response to the corneal injury. Progressive invasion of the cornea by the PMNs and the phagocytes increases the size of the ulceration, due to release of various lytic enzymes by the microbes.

This leads to necrosis and sloughing of the epithelium, Bowman’s membrane and the involved stroma (Fig. 2.3). The walls of the active ulcer project due to the swelling of the lamellae by imbibition of fluid. Ulceration may progress further by lateral extension leading to diffuse superficial ulceration or by deeper penetration of infection leading to descemetocele formation and possibly corneal perforation.

**Stage 2: Regressive Stage**

The termination of the progressive stage and the onset of the regressive stage is brought by the natural host defense mechanisms (humoral antibody response and cell mediate immune defenses) and the anti-microbial treatment. There is an improvement in the symptomatology and clinical signs. A line of demarcation forms around the ulcer so that the margin and floor of the ulcer become more smooth and transparent. The line of demarcation consists of leukocytes that neutralize and eventually phagocytose the offending organism and the necrotic cellular debris. The digestion of the necrotic material may cause an initial enlargement of the ulcer.

This process may be accompanied by superficial vascularization.

**Stage 3: Healing Stage**

The process of epithelialization starts to occur at this stage. The histiocytes and keratocytes convert to fibroblasts so that the scar tissue is formed. Vascularization occurs towards the ulcer site, which further promotes healing as a result of influx of fibroblasts and antibodies. When the healing is complete, the vessels regress and become “ghost vessels” which may be visualized by indirect illumination.

The degree of scarring from healing varies according to the depth of involvement. Bowman’s membrane does not regenerate and is replaced by fibrous tissue, which over a period of time becomes less dense, especially in young patients. The process of cicatrization occurs due to regeneration of collagen and the formation of fibrous tissue. Since the newly formed fibers are not laid down in a regular manner as in normal corneal lamellae, a scar is formed which causes the light to be refracted irregularly.

**SEQUEL AND COMPLICATIONS**

Corneal ulcers involving the superficial lamellae generally heal by varying degrees of scarring depending on the severity of inflammation. However, if the infection is severe, there may be thinning, formation of a descemetocoele ectatic cicatrix or perforation.

**Corneal Opacification**

Depending on the depth of the corneal ulceration, different types of corneal opacities may occur that is, nebular, macular (> 50% involvement) or leukomatous (> 75% involvement) (Figs 2.4A to D).

**Descemetocoele**

Some corneal ulcers, especially due to Pneumococci extend rapidly in depth so that the entire thickness of the cornea except Descemet’s membrane or few isolated corneal lamellae are spared. The Descemet’s membrane like any other elastic membrane offers resistance to the inflammatory process, but is unable to withstand the intraocular pressure and therefore herniates through the corneal ulcer as a transparent membrane called as descemetocoele or a keratocele (Fig. 2.5). This is often
Figure 2.5: Descemetocele formation with perforation

Figure 2.6: Perforated corneal ulcer with pseudocornea formation

Figures 2.4A to D: Healing corneal ulcer with end stage leukomatous corneal opacity
surrounded by a white cicatricial ring and under the influence of a raised intraocular pressure may eventually rupture.

Perforation

Perforation of corneal ulcer occurs due to sudden exertion by the patient such as coughing, sneezing, straining or spasm of the orbicularis muscle. An increase in intraocular pressure occurs due to these maneuvers so that the weak floor of the ulcer gives way. When an ulcer perforates, the aqueous suddenly escapes and the intraocular pressure falls to the atmospheric levels. Subsequently, the lens iris diaphragm moves forward and adheres to the back of the cornea. Due to the decreased intraocular pressure, the pain is alleviated; extension of the ulcer decreases and the process of scar formation is initiated.

If the perforation is small, the iris is plugged to the back of the cornea, adhesions from the iris get organized and the scar tissue is formed which is called as “pseudocornea” (Fig. 2.6). The iris, which is plastered at the back of the cornea, allows anterior chamber to form and hence aqueous is secreted.

If the perforation is large, the iris prolapses out of the site of perforation; in cases of longstanding of iris prolapse, fibrin and exudates deposition occurs on the surface, thinning of the iris stroma occurs and the black pigmented epithelium becomes visible. Thus any adherence of iris tissue to the back of cornea, which is subsequent to a perforated corneal ulcer, is called as a corneo-iridic scar (Fig. 2.7).

In very large perforations, only a small rim of the cornea remains and the total prolapse of the iris and the lens may occur (Fig. 2.8). If the perforation occurs suddenly, the suspensory ligament of the lens gives way, causing subluxation of the lens, anterior dislocation and spontaneous expulsion of the lens and the vitreous through the perforation (Figs 2.9A and B).

Ectatic Cicatrix

Due to the presence of anterior synechiae and plugging of the iris, adherent leukoma is formed and this leads to secondary glaucoma. The cicatricial tissue is too weak to support this raised intraocular pressure and hence the cicatrix becomes ectatic. An ectatic cicatrix into which the iris is incarcerated is called as the anterior staphyloma, which may be partial or total. It is so called due to the lobulated appearance (Fig. 2.10).

Corneal Fistula

If the perforation occurs near the pupillary margin, the iris becomes adherent to the back of the cornea and the aperture is filled with the fibrin and the exudates. As the anterior chamber reforms, the aperture is subjected to repeated strain, so that a permanent opening forms which is called as the corneal fistula (Fig. 2.11).

Hemorrhage

The sudden decrease in the intraocular pressure when perforation occurs dilates the intraocular blood vessels, which may rupture causing an intraocular hemorrhage. Rupture of retinal vessels may give rise to vitreous hemorrhage, choroidal, a subretinal or subchoroidal hemorrhage. It may be so profuse that it may lead to expulsion of the intraocular components leading on to expulsive hemorrhage.
The organisms, which are causing the ulceration of the cornea, may gain access to the interior of the eye as a result of perforation and cause purulent iridocyclitis, endophthalmitis (Fig. 2.12) and even panophthalmitis.

References

Introduction

The various microorganisms which can cause infectious keratitis can be classified into eukaryotic and prokaryotic organisms (Table 3.1). The eukaryotic organisms include the relatively complex cells such as protozoa and the fungi and the prokaryotic organisms are more primitive cells, which include the filamentous bacteria, true bacteria, spirochaetes, mycoplasma and rickettsiae and chlamydiae.

Protozoa

Protozoa are unicellular organisms which exist in two morphologic phases. Examples which cause ocular infections include the Acanthamoeba and the Microsporidia.

<table>
<thead>
<tr>
<th>TABLE 3.1 Classification of microorganisms causing infectious keratitis</th>
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<tr>
<td><strong>EUKARYOTES</strong></td>
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<tr>
<td>Protozoa</td>
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<tr>
<td>Sporozoan: Toxoplasma</td>
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<tr>
<td>Amoeba: Entamoeba, Naegleria, Acanthamoeba</td>
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<tr>
<td>Microsporidia</td>
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<td>Fungi</td>
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<tr>
<td>Mouldlike: Aspergillus</td>
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<tr>
<td>Yeastlike: Candida</td>
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<tr>
<td>Dimorphic: Histoplasma, Blastomyces, Coccidioides</td>
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<td>True yeasts: Cryptococcus</td>
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<tr>
<td><strong>PROKARYOTES</strong></td>
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<tr>
<td>Filamentous bacteria</td>
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<tr>
<td>Actinomyces, Nocardia, Mycobacterium, Streptomyces</td>
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<tr>
<td>True bacteria</td>
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<tr>
<td>Gram-positive Bacilli and Cocci</td>
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<tr>
<td>Gram-negative Bacilli and Cocci</td>
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<tr>
<td>Spirochaetes</td>
</tr>
<tr>
<td>Borrelia, Treponema, Leptospira</td>
</tr>
<tr>
<td>Mycoplasma</td>
</tr>
<tr>
<td>Rickettsiae and Chlamydia</td>
</tr>
</tbody>
</table>

Acanthamoeba

Primarily, Acanthamoeba is the protozoa, which can cause keratitis. The pathogenic species of Acanthamoeba include Acanthamoeba castellanii, Acanthamoeba polyphaga, Acanthamoeba culbertsoni, Acanthamoeba palestinensis, Acanthamoeba astronyxis, Acanthamoeba hatchetti, Acanthamoeba rhysodes, Acanthamoeba divionesis, Acanthamoeba equina, Acanthamoeba lugdunensis, and Acanthamoeba griffini.

Acanthamoeba are ubiquitous organisms and have been isolated from soil, water (including natural and treated water), air, and dust. They are free living, pathogenic amoeba. Most persons are exposed to this organism during their lifetime, as 50-100 percent of healthy people have serum antibodies directed against Acanthamoeba.

The life cycle consists of 2 stages: a trophozoite (which is 14-45 microns in diameter) and a cyst (which has a double-layered wall with a diameter of 10-25 microns). The trophozoites are motile and usually have one nucleolus and huge cytoplasmic vacuoles and feeds on bacteria (Fig. 3.1). In unfavorable conditions the trophozoites encyst. In unfavorable conditions the trophozoites encyst. The cyst has a double wall, which is made of cellulose (Fig. 3.2). The cyst is resistant to alterations in temperature, pH, osmolarity and antimicrobial agents.

Microsporidia

Microsporidia are eukaryotic, spore forming obligate intracellular parasites. Microsporidial keratitis occurs in two forms keratoconjunctivitis is usually seen in immunocompromized individuals or in contact lens wearers, mostly by genus Encephalitozoon while Nosema and Microsporidium cause the stromal keratitis which is generally seen in the immunocompetent host. Most infections are transmitted by feco-oral route but corneal infections occur due to direct inoculation. These
organisms stain poorly with the routine stains such as the Gram’s, Giemsa, Gomori methanamine silver, Periodic acid Schiff and immunofluorescent techniques. However, they stain well with calcofluor white stain.4

**Mycology**

Keratomycosis is common in tropical climatic regions and is a common cause of corneal ulcer. Fungi are opportunistic organisms and rarely affect intact cornea, but in a compromised or immunosuppressed state such as ocular surface disease, topical steroid use or trauma with vegetable matter particularly, they become pathogenic.

The cell wall of the fungi is primarily made up of polysaccharide (80 to 90%) and the remainder is protein or lipid.

Keratomycotic fungi can be broadly classified into filamentous fungi, yeasts, or dimorphic fungi (Table 3.2).

More than 105 species of fungi classified in 56 genera have been reported to cause oculomycosis. Fungi are saprophytic, and/or pathogenic organisms. Saprophytic fungi obtain their nutrients from decaying organic matter, whereas pathogenic fungi feed on living cells. Pathogenic fungi are actually saprophytic microbes, which cause disease in humans. Many fungi associated with ocular infections are saprophytic.

Fungal isolates can be classified into four groups: Moniliaceae, which are nonpigmented filamentary fungi including *Fusarium spp* and *Aspergillus spp*; Dematiaceae, which are pigmented filamentary fungi including *Curvularia spp* and *Lasiodiplodia spp*; yeasts, which include *Candida spp*; and other fungi.

**TABLE 3.2**

<table>
<thead>
<tr>
<th><strong>Fungal organisms causing keratitis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. FILAMENTOUS</strong></td>
</tr>
<tr>
<td><strong>A. SEPTATED</strong></td>
</tr>
<tr>
<td>1. Nonpigmented</td>
</tr>
<tr>
<td><em>Fusarium</em>: Solani, oxysporum, moniliforme, episphaeria</td>
</tr>
<tr>
<td><em>Aspergillus</em>: fumigatus, flavus</td>
</tr>
<tr>
<td><em>Acremonium</em> (Cephalosporium)</td>
</tr>
<tr>
<td><em>Paecilomyces</em></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
</tr>
<tr>
<td>2. Pigmented (Dematiaceous)</td>
</tr>
<tr>
<td><em>Curvularia</em>: Senegalensis, verruculosa, pallescens</td>
</tr>
<tr>
<td><em>Lasiodiplodia</em> theobromae</td>
</tr>
<tr>
<td><em>Alternaria</em></td>
</tr>
<tr>
<td><em>Cladosporium</em></td>
</tr>
<tr>
<td><em>Celltotrichum</em></td>
</tr>
<tr>
<td><em>Drechslera</em> (Helminthosporium)</td>
</tr>
<tr>
<td><strong>B. NONSEPTATED</strong></td>
</tr>
<tr>
<td><em>Rhizopus</em> (mucormycosis)</td>
</tr>
<tr>
<td><strong>II. YEAST</strong></td>
</tr>
<tr>
<td><em>Candida</em>: Albicans, parapsilosis, krusei, tropicalis</td>
</tr>
</tbody>
</table>

**FILAMENTOUS FUNGI**

Molds are filamentous fungi, which are multicellular organisms and possess hyphae and grow by apical extension or branching. They have a rigid cell wall composed of outer chitin and inner sterol containing cytoplasmic membrane. According to the presence or absence of cross walls in the hyphae the filamentous fungi are classified as septate or non-septate. Most fungal keratitis is caused by filamentous fungi which have septate hyphae.

Fungi with septate hyphae may be divided into non-pigmented fungi or pigmented fungi based upon
whether the hyphae are pigmented or non-pigmented on the culture media. Filamentous septate fungi can thus be broadly classified into:

i. Moniliaceae (light colored fungi like *Fusarium* and *Aspergillus* species)

ii. Dematiaceae (dark colored fungi such as *Alternaria* and *Curvularia*).

**YEASTS**

Yeasts are unicellular fungi, represented by *Candida*, and are characterized by an oval or round blastoconidium. Yeasts reproduce by budding and are characterized by the presence of pseudo hyphae (Fig. 3.3). The phase, which has pseudo hyphae, is the most virulent phase. The cell walls of pseudo hyphae have constrictions and are not parallel to each other unlike the hyphae.

*Candida* species especially *Candida albicans* commonly causes majority of the cases of keratitis. In culture they form smooth creamy white colonies resembling staphylococci in the early phases of growth. The presence of budding yeasts in corneal scrapings is diagnostic for *Candida*. This organism produces both true hyphae and pseudo hyphae. Both yeast and hyphal forms can be seen in corneal scrapings. Candida colonies are white to tan and opaque with smooth, round, flat contour and pasty consistency. They have a distinctive fruity odor, which aids in easy identification.

**DIMORPHIC FUNGI**

Some fungi appear as yeasts *in vivo* at 37°C and molds in the environment at 25°C. They are called as dimorphic fungi. These include *Blastomyces*, *Coccidioides*, *Histoplasma* and *Sporothrix*.

**Moniliaceae**

**ASPERGILLUS**

This is a common contaminant in hospital air. *Aspergillus fumigatus* is the most commonly isolated species, while *Aspergillus flavus* and *Aspergillus niger* may also cause keratitis. The Aspergillus fungi are readily recognized by their morphology (Fig. 3.4). The conidospore with its swollen terminal end is surrounded by a flask shaped sterigmata, each of which produces long chains of conidia that radiate from the terminal end (Fig. 3.5). Their hyphae are septate and branch dichotomously. In infections progressing rapidly they are more uniform but in more indolent infections the hyphae may be irregular in shape.

Colonies of *A. fumigatus* are at first white but as spores are produced they become velvet green due to pigmentation of conidia. *A. niger* on the other hand, turns completely black as they undergo sporulation.

**FUSARIUM**

*Fusarium* causes localized infection after trauma in otherwise healthy patients. *Fusarium solani* is the most common species. It has sickle shaped macroconidia and clusters of fusiform microconidia (Fig. 3.6). *Fusarium* colonies are white initially and later acquire a buff coloration. A range of color pigments from yellow to red to purple is produced on the undersurface of the colony (reverse pigmentation).
DEMATIACEAE

Dematiaceous fungi have melanin in their hyphal walls and hence are dark pigmented (olive, brown or black) when viewed under a microscope. Only 27 percent of corneal ulcers caused by dematiaceous fungi appear to be darkly pigmented macroscopically. *Curvularia* (Fig. 3.7), *Alternaria* (Fig. 3.8) and *Cladosporium* belong to this category.

Bacteriology

Bacteria are unicellular prokaryotic organisms. The cornea does not generally have any colonizing bacteria. Bacteria from eyelids may contaminate the ocular surface and cause corneal ulcer.

Pathogenic bacteria consist of virulence factors, which is responsible for infectious keratitis. These include specific antigens, proteolytic enzymes, hemolysins and toxins. In general non-pathogenic bacteria do not cause keratitis except in compromised conditions when they gain access to the cornea.

The classical differential system of staining the bacteria divides the bacteria into gram-positive and gram-negative bacteria depending on the presence of peptidoglycan and lipid layer of the cell wall. Gram-positive bacteria are more frequently isolated as compared to the gram-negative bacteria in the ratio of approximately 60 to 40 percent. Apart from this there are other stains such as acid-fast, which may be, used to characterize the mycobacteria species.
**Specific Bacteria**

**GRAM-POSITIVE BACTERIA**

Gram-positive bacteria have a high content of peptidoglycan and a low content of lipid compared to gram-negative bacteria. When the lipid layer is dissolved, crystal violet and iodine form a complex on the cell wall that appears blue under the microscope, depicting gram-positive bacteria.

The most common organisms which infect the eye are the *Staphylococcus aureus*, Coagulase negative Staphylococci, *Streptococcus pneumoniae* and *Streptococcus viridans*.

*Staphylococcus aureus*

*Staphylococcus aureus* are gram-positive cocci (0.5 to 1.5 μm), which are seen under the microscope in pairs or grape like clusters and grow as routine culture media within 18 to 24 hours. On blood agar, they appear as golden hemolysis (clear area). They are non-motile, non-spore forming, facultative anaerobes and are usually encapsulated. They are coagulase and catalase positive. *Staphylococcus* species resistant to oxacillin agents constitutes the methicillin resistant *Staphylococcus aureus* (MRSA).

*Coagulase Negative Staphylococci*

The genus *Staphylococcus* consists of 32 species out of which 3 are coagulase positive and the rest are coagulase negative. Coagulase negative *Staphylococcus* are normal inhabitants of human body especially the skin, mucous membrane and the eyelid margins.

Coagulase negative *Staphylococcus* are microscopically similar to *Staphylococcus aureus* and appear in pairs or grape like clusters, but on blood agar isolation, they appear as white to grayish colonies within 24 to 48 hours of incubation. These bacteria are non-motile, non-spore forming, facultative anaerobes, usually non-encapsulated, catalase positive. The most common bacteria implicated in bacterial keratitis is negative coagulase *Staphylococcus*.

*Antibiotic susceptibility*: Just like *Staphylococcus aureus* the resistance rate of 55 percent has been noted to fluoroquinolones in coagulase negative *Staphylococcus*. They are susceptible to vancomycin, bacitracin and chloramphenicol.

**MICROCOCCUS SPECIES**

They are gram-positive cocci, which may be present as saprophytes and inhabit the eyelid margins. They appear on blood agar as yellow distinct colonies.

*Streptococcus* and Related Bacteria

Streptococci appear microscopically as gram-positive cocci (Fig. 3.9), usually cocoid or coccobacilli and appear in chains of cocci on broth medium. They may be present as normal inhabitants especially in children. Streptococci are distinguished from *Staphylococcus* and *Micrococcus* species by negative catalase reaction. *S.pneumoniae* is less virulent as compared to *S.viridans*. They are responsible for corneal ulcers, recalcitrant graft infections and infectious crystalline keratopathy.

*Streptococcus* species are differentiated by their hemolysis patterns on blood supplemented agar media into the following:

1. Alpha-hemolytic streptococci which partially lyse the red blood cells and a greenish halo appears around a colony. Examples of alpha-hemolytic streptococci include *Streptococcus pneumoniae* and *Streptococcus viridans*. 

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*Figure 3.9: Gram-positive pneumococci (Courtesy: Dr H Sheorey, Dept. of Microbiology, St Vincent Hospital, Melbourne)*
2. Beta-hemolytic streptococci lyse red blood cells completely and a clear halo appears around a colony. An example of beta-hemolytic streptococci is *Streptococcus pyogenes* which when identified from cornea is pathogenic.

3. Gamma-hemolytic or non-hemolytic streptococci which have no hemolysis pattern around a colony.

**Antibiotic Susceptibility**

They exhibit high resistance to polymyxin. They are susceptible to cefazolin, bacitracin, chloramphenicol and sulphacetamide. The *in vitro* susceptibility of *Streptococcus* species to fluoroquinolones is low.

**Enterococcus**

*Enterococcus* species are gram-positive ovoid cocci or coccobacilli and are isolated or broth media with or without red blood supplementation. *Enterococcus fecalis* is the most common species isolated from the cornea which can cause corneal ulcer.

**Diphtheroids**

Diphtheroids appear microscopically as gram-positive pleomorphic rods. They are aerobic non-spore formers. The most common diphtheroid isolated from the compromised corneas include *Corynebacterium* and *Propionibacterium*. They grow well on blood-supplemented media, in a CO$_2$ atmosphere but it may require 24 to 48 hours extra for the colonies to appear. Enriched thioglycollate, liquid broth is a good medium for the colonies to appear. Diphtheroids are known to occur as saprophytes, although in compromised conditions they may also cause infectious keratitis.

*Corynebacterium diphtheriae* and *Listeria monocytogenes* are corneal pathogens, which can breach the intact epithelium without prior trauma.

**Antibiotic susceptibility:** They are susceptible to vancomycin, chloramphenicol, ofloxacin and partially to cefazolin. Diphtheroids demonstrate a good *in vitro* susceptibility to most antibiotics except to trimethoprim.

**Mycobacterium**

Mycobacteria are obligate parasites and opportunistic pathogens. They are gram-positive bacteria which are slightly curved or straight bacilli. They appear as “ghosts” or beaded gram-positive rods on Gram stain. *Mycobacterium chelonae* and *Mycobacterium fortuitum* are responsible for keratitis after refractive surgery. On blood agar and chocolate agar plates, the colonies may take a week to appear. Routine mycobacterial isolation medium such as Löwenstein-Jensen medium is more definitive for isolation or organisms.

When mycobacteria are suspected to cause keratitis, special acid-fast stains should be used to stain them as they have a cell wall with high content of lipid which resists Gram staining.

**Antibiotic susceptibility:** They are susceptible to clarithromycin and amikacin.

**Bacillus**

*Bacillus* species are large, gram-positive rods but may sometimes be gram-variable. They are spore bearing, catalase positive, motile and grow aerobically or as facultative anaerobes. The most important corneal pathogens are *Bacillus cereus* and *Bacillus anthracis*, which appear as large, grainy, dry beta-hemolytic colony on agar media. Most organisms are opportunistic pathogens.

**Antibiotic susceptibility:** They are susceptible to aminoglycosides, fluoroquinolones and sulphacetamides.

**Nocardia**

*Nocardia* species belong to the group of actinomycetes and appear as gram-positive, branching, filamentous bacteria. They are aerobic, non-motile, partially acid-fast and appear as white, tiny, dry colonies.

They are associated with infections after trauma, contact lens wear and following laser *in situ* keratomileusis (LASIK) surgery. The pathogenic species are *Nocardia asteroides* and *Nocardia brasiliensis*.

**Antibiotic susceptibility:** They are susceptible to sulphacetamide, trimethoprim – sulframethoxazole and amikacin.

**GRAM-NEGATIVE BACTERIA**

Gram-negative bacteria appear red because the lipid layer is not removed by the decolorizing step. A blue crystal violet complex cannot form in the cell wall and safranin counter stains the bacteria (Fig. 3.10).

The common gram-negative bacteria, which infect cornea include *Pseudomonas aeruginosa*, *Serratia marce-
scens, Moraxella species and Haemophilus species. Generally gram-negative infection occurs due to bacilli. Rarely, gram-negative cocci may cause infection such as in cases of Neisseria gonorrhoeae, Neisseria meningitides and Branhamella catarrhalis.

**Pseudomonas aeruginosa**

*P. aeruginosa* are aerobic bacteria, which appear as gram-negative bacilli. It grows between 30-37°C and on trypticase broth supplemented with 5 percent sheep blood appear grayish or greenish, metallic-appearing as gelatinous colonies. These colonies have a grape-like odor. The colonies of *P. aeruginosa* are oxidase positive.

The infections due to this organism are generally fulminant because of the presence of virulence factors (exotoxin A, proteolytic enzymes), presence of pili, which attach to the cells and production of alginate, which is a polysaccharide polymer, which inhibits phagocytosis.

Contact lens wearers, debilitated patients, patients with systemic diseases and in intensive care units are at risk for *Pseudomonas* keratitis.

**Serratia marcescens**

*Serratia marcescens* is an opportunistic pathogen, which along with corneal epithelial breakdown due to trauma or contact lenses can lead to corneal ulceration. On Gram stain they appear as coccobacilli and its colonies are reddish in color. They are a frequent contaminant of contact lenses and contact lens solutions.

**MORAXELLA**

Microscopically, they appear as gram-negative brick shaped diplobacilli (Fig. 3.11). They cause chronic conjunctivitis and acute corneal ulceration. *Moraxella lacunata* is the commonest species involved. The colonies appear grayish in color and appear pitted.

**HAEMOPHILUS**

Haemophilus appear as tiny gram-negative coccobacilli. These bacteria require factor V and factor X for their growth and hence grow better on chocolate agar than on blood agar. The colonies appear as grayish in color and have a musty smell.

**GLUCOSE FERMENTERS AND NON-FERMENTERS**

Glucose fermenters include *Stenotrophomonas* and *Alcaligenes* species and glucose non-fermenters include *Escherichia coli*, *Enterobacter*, *Klebsiella* and *Proteus* species.

**Stains and Culture Media**

Various methods have been used to identify the organisms from a case of infectious keratitis. These include various stains to identify the morphology of the inciting organisms and culture media, which are used to grow these organisms.

Antibiotic susceptibility: They are susceptible to fluoroquinolones and aminoglycosides and have intermediate susceptibility to chloramphenicol, trimethoprim and sulfasoxazole.
STAINING METHODS

The stains used to routinely identify various organisms include potassium hydroxide wet mount preparation, Gram’s stain, Giemsa stain and special stains such as Ziehl-Neelsen acid-fast stain, fluorochromatic stains and modified Grocott-Gomori methenamine-silver nitrate stain.

Potassium Hydroxide Wet Mount Preparation

We recommend simple microscopic examination of corneal scraping using 10 percent KOH preparation in all cases of suspected infectious keratitis. This is a simple test and can be performed in outpatient area and does not involve too much of cost.

The scraped material is spread out as thinly as possible with the help of spatula on the slide. One drop of 10 percent KOH solution is put on the scrapings and a slide cover is placed. The slide is examined under a microscope. The KOH helps in loosening the corneal stromal lamellae and exposing more fungal filaments. It also stains the filaments in a very light yellow color (Fig. 3.12). Ten percent KOH mount examined by conventional microscope is a useful test in helping identification of fungi and Acanthamoeba. The test has high sensitivity (92%) and a high specificity (96%).8,9

Gram’s Stain

Gram’s stain classifies the bacteria into two major groups based on the cell wall of the bacteria. Gram-positive bacteria retain the gentian violet-iodine complex and appear blue-purple, whereas the gram-negative bacteria lose their gentian violet–iodine complex with decolorization step and appear pink when counterstained with safranin. A 5 minute Gram’s staining procedure as well as a 5 seconds Gram’s staining procedure is also available. We prefer to use the 5 minutes Gram’s staining procedure (Table 3.3).

Overall, Gram’s stain is accurate in 61 percent of cases of bacterial keratitis. If performed correctly, Gram’s stain identifies the organism correctly in up to 75 percent of the cases caused by a single organism and in 37 percent cases of polymicrobial keratitis.10

Giemsa Stain

Giemsa stain is one of the Romanowsky type stain, which uses eosin, methylene blue and azure dyes. It stains the DNA in the nuclei of the human cells and cytoplasmic RNA in the lymphocytes.

The Giemsa stain is usually used to determine the type of inflammatory cells present. We do not recommend its use routinely. This stain differentiates bacteria from fungi, and also identifies chlamydia inclusion bodies and cysts and trophozoites of Acanthamoeba species. It identifies the normal and inflammatory cells. With Giemsa technique the bacteria appear dark blue in color. The yeast cells and fungal hyphae absorb the stain and appear purple or blue while the cell walls and the septations do not stain. The staining solution should be freshly prepared daily.

The conventional Giemsa stain takes 60 minutes to perform (Table 3.4), although a rapid 15 minutes modi-
Ziehl-Neelsen Acid-fast Stain

Special stains include the use of carbol-fuchsin or Ziehl-Neelsen acid-fast stain for identification of suspected Mycobacteria, Actinomyces or Nocardia. Mycobacteria are acid-fast, Nocardia stain variably, whereas Actinomyces are non-acid fast.

The principal of this stain is based on the resistance of the mycobacterial species and certain strains of Nocardia to decolorization by strong mineral acids after staining with basic carbol fuchsin. This resistance is due to the presence of intact cells that contain specific lipid unsaponifiable wax fraction.

Fluorochromatic Stains

Fluorochromatic stains such as acridine-orange and calcofluor-white require the use of an epifluorescence microscope to visualize the organisms and the cells.

Calcofluor White

Calcofluor white binds to chitin and cellulose. Because the cell walls of the yeast and filamentous fungi are composed of chitin and cellulose, these organisms stain bright green with calcofluor white under epifluorescent microscope. The cysts of Acanthamoeba likewise also have chitin and cellulose and also stain bright green. The trophozoites of Acanthamoeba stain reddish-orange in color.

Acridine Orange

The acridine orange is a chemofluorescent dye, which stains fungi and bacteria yellow-orange against a green background when the pH is acidic and a fluorescence microscope is used. It is a valuable stain as it identifies gram-positive and gram-negative bacteria, yeast and hyphal forms of fungi and both the trophozoite and cyst form of Acanthamoeba. The Gram’s stain can be directly done on a slide where prior staining with acridine orange has been done without destaining. The acridine orange stains accurately predicts culture results in 71 to 84 percent of cases in comparison to 62 to 79 percent for Gram’s stain.

Modified Grocott-Gomori Methenamine-Silver Nitrate Stain

The Grocott-Gomori methenamine-silver nitrate stain is a histopathologic stain used to show fungi and has been modified for the examination of corneal scrapings. For fungal infections, this stain is more reliable than the Gram’s, Giemsa, or KOH stain. The staining procedure is described in the Table 3.5. The specimens should be spread onto gelatin-coated slides, which can be prepared by spreading a drop of warm gelatin (1%) over the slide to form a thin coat. The slides can be stored in the freezer at -20 to 0°C until used. The working solution of methenamine-silver nitrate must be made fresh before each use. With the methenamine-silver nitrate stain, fungus cell walls and septa black and can be easily seen against the background, which is a faint transparent green (Fig. 3.13).

Procedure

- Spread the specimen on a gelatin-coated slide to form a thin smear.
• Fix the slide in methyl alcohol for 5 minutes.
• Oxidize the slide in 5 percent chromic acid for 30 minutes.
• Preheat the working methenamine-silver nitrate solution to 58-60°C and place the oxidized slide in the heated solution for 20 minutes. The smear will be amber in color.
• Promptly rinse the slide in six changes of distilled water.
• Tone the slide in 0.1 percent gold chloride for 2-4 minutes (the gold chloride can be reused).
• Rinse in two changes of distilled water.
• To reduce the silver, place the slide in a 2 percent sodium thiosulfate solution for 2 minutes.
• Rinse in tap water.
• Counterstain briefly (40-60 seconds) using a fresh solution of 1:5 stock light green in distilled water.
• Allow to air dry.

The summary of various stains used to diagnose different types of organisms is given in Table 3.6.

### TABLE 3.6
Diagnostic stains used for preparation of smear

<table>
<thead>
<tr>
<th>Type of stain</th>
<th>Organism</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Bacteria</td>
<td>Gram-positive-purple</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram-negative-pink</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>Bacteria</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td></td>
<td><em>Acanthamoeba</em></td>
<td></td>
</tr>
<tr>
<td>Calcofluor white</td>
<td>Fungi</td>
<td>Bright green</td>
</tr>
<tr>
<td></td>
<td><em>Acanthamoeba</em></td>
<td>Bright green</td>
</tr>
<tr>
<td></td>
<td>cysts</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acanthamoeba</em></td>
<td>Reddish orange</td>
</tr>
<tr>
<td></td>
<td>trophozoites</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mycobacteria</td>
<td></td>
</tr>
<tr>
<td>Acid-fast</td>
<td>Mycobacteria</td>
<td></td>
</tr>
</tbody>
</table>

### Nutrient agar

Nutrient agar is the simplest medium used in most laboratories. It is made by adding 2 percent agar to nutrient broth (Fig. 3.14).

Nutrient agar contains beef extract (which supplies carbohydrates, organic nitrogen, salts, vitamins), peptone (enzymatic digest of certain proteins; provides readily available nitrogen), agar (extract of marine algae which is used as a solidifying agent) and water. It has a pH of 6.8.

### Blood agar

The agar is derived from seaweed with the addition of 5 to 10 percent red blood cells, which provide nutrients,
as well as an index of hemolysis (Fig. 3.15). This is a standard medium for isolation of aerobic bacteria at 35°C. It also supports the growth of saprophytic fungi and *Nocardia* at room temperature.

**Chocolate agar**

Chocolate agar is incubated with 10 percent carbon dioxide to isolate facultative organisms. It is prepared by heat denaturation of blood to 56°C to provide human and diphosphopyridine nucleotide for the growth of organisms such as *Haemophilus*, *Neisseria* and *Moraxella*.

**Thioglycolate broth**

This is a liquid media, which is incubated at 35°C for and is used for the isolation of aerobic and anaerobic bacteria. It contains a sulphydryl compound which acts as an oxygen-reducing agent to facilitate recovery of anaerobic bacteria. Thiol is a variation of thioglycollate broth and contains special complexes and 0.1 percent semisolid agar to prevent convection currents and promote the growth of aerobic bacteria as well as obligate and facultatively anaerobic organisms.

**Sabouraud agar**

This consists of Sabouraud glucose and peptone agar and is a non selective media for opportunistic fungi. Yeast extract is used to improve the nutrition and an antibiotic such as gentamicin is added to inhibit the bacterial contamination (Fig. 3.16). The medium should not contain cycloheximide as this inhibits the growth of the saprophytic fungi.

Sabouraud agar is incubated at room temperature and is used for isolation of fungi and *Nocardia*.

**Löwenstein-Jensen Medium**

Mycobacteria are specifically isolated on Löwenstein-Jensen media. Glycerol and egg mixture which are added to this media provide fatty acids and protein required for the metabolism of mycobacteria. The coagulated egg albumin provides a solid medium for inoculation purposes. It also contains malachite green as an inhibitor to microorganisms other than acid-fast bacilli. Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

**Thayer-Martin Medium**

This is a special, selective, chemically enriched chocolate agar, which is used to isolate *Neisseria gonorrhoeae* by suppressing the growth of other inhibitory fungi and bacteria.

**Brain-heart Infusion (BHI) broth**

Brain-heart infusion broth with neopeptone is incubated at room temperature and is used to enhance the isolation of filamentous fungi and yeasts and less frequently *Bacillus* species.

**Robertson Cooked Meat Medium**

Robertson cooked meat medium provides a favorable environment for the growth of anaerobes. The muscle
tissue is a source of amino acids and reducing substances, particularly glutathione, which permits the growth of strict anaerobes. Glucose, Hemin and Vitamin K1 is supplemented with yeast extract, to enhance the growth of anaerobic microorganisms (Fig. 3.17). Growth is indicated by turbidity and, with some organisms, by the presence of gas bubbles in the medium. Disintegration and blackening of the meat particles indicates proteolysis.

References

Medical therapy forms the first line of treatment of infectious keratitis. The drugs used to treat microbial keratitis include antimicrobial agents, cyclopenters, antialglaucma medications and adjuvants.

**Antibacterial Agents**

The various anti-bacterial agents can be given by topically, subconjunctivally or by systemic route in a case of bacterial keratitis (Table 4.1). Topical route using concentrated or fortified antibiotics is generally preferred as higher concentration of the antibiotics are achieved by this route.

Fortified antibiotics are prepared by adding the required amount of parenteral agent to an artificial tear solution or to a commercially prepared topical solution.

Subconjunctival injections of antibiotic can achieve therapeutic levels due to leakage from the injection site into the tear film and direct penetration into the adjacent tissue. However, since there is no added benefit over topical administration we do not use subconjunctival antibiotics routinely.

Systemic therapy is usually less effective in achieving therapeutic drug levels and carries the risk of systemic toxicity. These are usually used to treat corneal infections in cases of scleral involvement, perforated corneas or impending perforations disease.

**ANTIBACTERIAL AGENTS**

**Beta Lactam Antibiotics**

Penicillin is the prototype drug of this group. They act by interfering in the synthesis of the bacterial cell wall proteoglycans. Topical beta lactam antibiotics are never available commercially they are somewhat unstable in solution and tend to breakdown in days or weeks.

**Cephalosporins**

Like penicillins, cephalosporins contain a beta lactam ring which is required for its bactericidal action. The nucleus of the cephalosporins is 7 aminocephalosporanic acid, which is resistant to the action of penicillinases produced by staphylococci.

Cefazolin is primarily effective against gram-positive organisms.

Ceftazidime is a third generation cephalosporin, which acts against Pseudomonas species. It also has some activity against gram-positive organisms. It is particularly useful in cases of Pseudomonas keratitis.

<table>
<thead>
<tr>
<th>Gram’s stain</th>
<th>Topical</th>
<th>Subconjunctival</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive cocci</td>
<td>Cefazolin, 50 mg/mL</td>
<td>Cefazolin, 100 mg</td>
<td>Cefazolin, 200 mg/kg/day</td>
</tr>
<tr>
<td>Gram-negative cocci</td>
<td>Penicillin, 100,000 U/mL</td>
<td>Penicillin, 1 million U</td>
<td>Penicillin, 2- 6 million U q 4 hr</td>
</tr>
<tr>
<td>Gram-positive rods</td>
<td>Gentamicin, 14 mg/mL</td>
<td>Gentamicin, 20- 40 mg</td>
<td>Gentamicin, 3 mg/kg/day</td>
</tr>
<tr>
<td>Gram-positive filaments</td>
<td>Penicillin, 100,000 U/mL</td>
<td>Penicillin, 1 million U</td>
<td>Penicillin, 2- 6 million U q 4 hr</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>Cefazolin, 50 mg/mL, and gentamicin, 14 mg/mL</td>
<td>Cefazolin, 100 mg, and gentamicin, 20- 40 mg</td>
<td>Cefazolin, 200 mg/kg/day, and gentamicin, 3 mg/kg/day</td>
</tr>
<tr>
<td>Acid-fast bacilli</td>
<td>Amikacin, 10 mg/mL</td>
<td>Amikacin, 25 mg</td>
<td>Amikacin, 5 mg/kg/day</td>
</tr>
</tbody>
</table>
which are resistant to aminoglycosides or fluoroquinolones.2

**Tetracyclines**

Tetracyclines are broad-spectrum bacteriostatic antibiotics. Various forms of tetracycline are available, including chlortetracycline (topical), oxytetracycline, doxycycline, minocycline, and tetracycline. They act by inhibiting bacterial protein synthesis by binding to the 30-S ribosomes.

They are active against gram-positive organisms, gram-negative bacteria, mycoplasma, chlamydia and amoeba. They are not effective against *P. aeruginosa*, Bacteroides species, or group B streptococci. The various microorganisms acquire a plasmid-mediated resistance to tetracycline.

**Glycopeptides**

Vancomycin is a glycopeptide, which is effective penicillin-resistant Staphylococci. It inhibits the synthesis of peptidoglycan polymers during bacterial cell wall formation. It is active against gram-positive bacteria and remains one of the most potent agent against methicillin resistant Staphylococcus aureus and coagulase negative Staphylococci. Vancomycin should be reserved for cephalosporin-resistant Staphylococci. Vancomycin is effective against Streptococci and also against gram-positive bacilli such as Clostridia, Corynebacteria, Bacillus, *L. monocytogenes*, Actinomyces, and lactobacilli.

**Aminoglycosides**

Aminoglycosides act particularly against, gram-negative bacilli. They have a selective affinity to bacterial 30-S and 50-S ribosomal subunits to produce a nonfunctional 70-S initiation complex that results in inhibition of bacterial cell protein synthesis. Unlike other antibiotics that impair protein synthesis, they are bactericidal. Aminoglycosides have a bactericidal activity against aerobic, gram-negative bacilli such as *Pseudomonas aeruginosa*. However, there is resistance developing against Pseudomonas to gentamicin, tobramycin and to some extent amikacin. For Pseudomonas keratitis an aminoglycoside may be combined with a third generation cephalexinor. For Nocardia keratitis amikacin is the drug of choice.3

**Macrolides**

Macrolides are bacteriostatic agents (e.g. erythromycin, tetracycline) that can suppress the growth of susceptible gram-positive cocci. These drugs cause inhibition of bacterial protein synthesis by reversibly binding to the 50-S ribosomal unit thereby preventing elongation of peptide chain in bacteria. Erythromycin acts both as a bactericidal and a bacteriostatic agent depending on the concentration of the drug and is effective against gram-positive and some gram-negative organisms. *S. pneumoniae* and *S. pyogenes* are highly susceptible. It is one of the least toxic and best tolerated antibiotic agents. However, its penetration into cornea is sub-optimal due to lack of solubility and bioavailability.

Newer macrolides such as azithromycin and clarithromycin have higher tissue concentrations and are more effective against *C. trachomatis*, and non-tuberculous mycobacteria where they are used topically.4

**Fluoroquinolones**

The fluoroquinolone agents are the latest class of antibacterials. Four generations of fluoroquinolones have evolved:
1. **First generation**: Nalidixic acid—rarely used
2. **Second generation**: Ciprofloxacin and ofloxacin
3. **Third generation**: Levofloxacin
4. **Fourth generation**: Moxifloxacin and gatifloxacin.

**Mechanism of Action**

Fluoroquinolone agents act by inhibiting the supercoiling of DNA by the enzyme DNA gyrase. This action is bactericidal and occurs during cell replication. Fluoroquinolones have the advantage of low toxicity due to the lack of DNA gyrase in mammalian cells. The available topical agents include norfloxacin (0.3%), ciprofloxacin (0.3%), lomefloxacin, fleroxacin, perflaxin, and ofloxacin (0.3%), moxifloxacin (0.5%) and gatifloxacin (0.3%).

Moxifloxacin and gatifloxacin are C8-methoxy fluoroquinolones, and demonstrate increased activity against gram-positive bacteria and atypical mycobacteria when compared to ciprofloxacin, ofloxacin and levofloxacin *in vitro* and *in vivo*.

The available formulations of gatifloxacin (Zymar, Allergan, Inc.) and moxifloxacin (Vigamox, Alcon Inc.) are 0.3 percent and 0.5 percent respectively. The
Pharmacology

chemical structures of the C8-methoxy fluoroquinolones add protection from bacterial resistance in addition to enhancement of bactericidal properties. The substitution of the methoxy group at the eighth carbon on the basic ring in both gatifloxacin and moxifloxacin reduces the likelihood of ocular pathogens developing resistance to these fluoroquinolones. The methoxy group allows binding of the antibiotics to two bacterial enzymes - DNA gyrase and topoisomerase IV. As a result, C8 methoxy fluoroquinolones require two simultaneous mutations for development of resistance, the chance of which are one in ten trillion. Previous generations of fluoroquinolones required only a single mutation. Moreover, a bulky side chain at the C7 position of these antibiotics makes it difficult for the antibiotics to reach out of the bacterial cells.

Spectrum of Activity

The fluoroquinolones have broad-spectrum of activity and are more active against gram-negative bacteria than gram-positive bacteria. They are active against enteric gram-negative rods, such as Haemophilus influenzae and Neisseria gonorrhoeae. The third generation fluoroquinolones are active against Staphylococcus aureus, Non-coagulase Staphylococi, and Pseudomonas aeruginosa. Ofloxacin and ciprofloxacin have a comparable spectrum of activity against gram-positive and gram-negative organisms.5

The pathogens with respond less to fluoroquinolones include Streptococcus Pneumoniae, S. Viridans MRSA, anaerobes and non-aerugines Pseudomonas.6,7

Side Effects

The side effects of the fluoroquinolones may occur locally or they may be systemic. The topical administration of ciprofloxacin has been associated with crystal deposits in the cornea. This occurs in approximately 20 percent of patients treated with ciprofloxacin in cases of bacterial keratitis (Fig. 4.1). This crystallization has not been seen with norfloxacin or ofloxacin, presumably due to their high solubility. The advantage of crystalline deposits is that it acts as a depot from which the drug is released. However the disadvantage is that its presence retards epithelialisation.

The systemic side effects of fluoroquinolone include toxicity, fever, rash, and nausea which occurs in 4 percent of patients. Occasionally patients have elevation of levels of liver enzymes. The drugs can crystallize in the urine, especially in patients who are dehydrated. Interstitial nephritis has been reported after high doses of ciprofloxacin. Insomnia and restlessness have occurred in elderly patients taking fluoroquinolones. Children should not be given quinolones because animal studies have shown crystal deposits in cartilage and hence the topical fluoroquinolones are not recommended for children younger than 2 years.

PREPARATION OF FORTIFIED TOPICAL ANTIBIOTICS

(Adapted from Basic Clinical and Science Course 2000-2001. American Academy of Ophthalmology)

1. Cefazolin 50 mg/ml or ceftazidime 50 mg/mL
   a. Add 9.2 mL of artificial tears to a vial of cefazolin, 1 g (powder for injection).
   b. Dissolve. Take 5 mL of this solution and add it to 5 ml of artificial tears.
   c. Refrigerate and shake well before instillation.

2. Tobramycin 14 mg/mL or gentamicin 14 mg/mL
   a. Withdraw 2 mL of tobramycin or gentamicin injectable vial (40 mg/mL).
   b. Add 2 mL to a tobramycin or gentamicin ophthalmic solution (5 mL) to give a 14 mg/mL solution.

3. Vancomycin 15 mg/mL, vancomycin 25 mg/mL or vancomycin 50 mg/mL
   a. Add 33 mL of 0.9 percent sodium chloride for injection (no preservatives) or artificial tears to a 500 mg vial of vancomycin to produce a solution of 15 mg/mL. Add 20 mL of 0.9 percent sodium chloride for injection (no preservatives) or
artificial tears to produce a solution of 25 mg/mL. Add 10 mL of 0.9 percent sodium chloride for injection (no preservatives) or artificial tears to produce a solution of 50 mg/mL.

b. Refrigerate and shake well before instillation.

4. Amikacin
   Intravenous formulation can be used (80 mg/2 cc ampules).
5. Trimethoprim/sulfamethoxazole
   16 mg/mL and 80 mg/mL commercial preparation can be used.

**Antifungal Agents**

**CLASSIFICATION OF ANTIFUNGAL AGENTS**

1. Polyenes
   i. Large polyenes
      Nystatin, amphotericin B
   ii. Small polyenes
      Natamycin
2. Azoles
   i. Imidazoles
      Miconazole, ketoconazole, clotrimazole
   ii. Triazoles
      Fluconazoles, itraconazoles
3. Pyrimidines
   Flucytosines

**POLYENES**

**Mechanism of Action**

Polyene antibiotics are produced from a Streptomyces species. They interact with cell membrane sterols present in fungi, mainly ergosterol, which causes increased permeability that leads to cell lysis. It is this binding to mammalian cell membrane cholesterol that is responsible for their toxicity.

Two mechanisms of action of the polyene antibiotics are known depending on the size of the antifungal molecule. Small molecules such as natamycin work by an all-or-none mechanism of action. They bind to the esters in the fungal cell wall, forming “blisters” and causing lysis of the cell. This action is not concentration dependent. The larger molecules, such as amphotericin, work by creating “pores” in the cell wall, allowing small ions such as potassium to leak out and causing imbalances in the osmotic gradient and eventual cell lysis. This mechanism of action is concentration dependent and may be altered by changes in the osmotic environment.

**Nystatin**

Nystatin was the first polyene antibiotic identified. It is not used routinely because of its corneal toxicity and poor ocular penetration.

**Natamycin**

Natamycin is a small semisynthetic tetraene and is considered the least toxic, the least irritating, and the most stable of all polyenes. It has broad spectrum of activity, especially against Fusarium species. It has low penetration in the presence of an intact epithelium. Surface debridement of the cornea increases its penetration. Since natamycin is used as a suspension, it can dry on the ocular surface forming white deposits on the cornea and adenexa. Natamycin can be toxic to the corneal and conjunctival epithelium, causing hyperemia and epithelial defects.

**Dosage**

It is available for topical use as a 5 percent suspension (Table 4.2). Topical therapy is given every hour for the first 48 to 72 hours, and treatment is usually continued on a tapering basis for 3 to 6 weeks depending on the activity of the keratitis.

**Amphotericin B**

Amphotericin B is a haptene polyene that is insoluble in water and degrades rapidly on exposure to light. It is active against Candida, Aspergillus and Cryptococcus. Its activity against the filamentous fungi is variable. It has good penetration in the cornea and aqueous in inflamed eyes. As with natamycin, its penetration increases with debridement of the cornea. Topical use causes punctate epithelial erosions and a greenish discoloration of the conjunctiva (Table 4.2). Even a small dose of 0.1 mg given subconjunctivally is extremely toxic. This can cause conjunctival nodules which are transient in nature and yellow discoloration which can be permanent.

**Dosage**

Amphotericin B is used as topical drops in concentration of 0.15 percent (Table 4.2). It has to be prepared fresh in dextrose. The mixture should be stored in dark bottles.
and stored under refrigeration at 2-8°C to prevent chemical degradation.

**AZOLES**

**Imidazoles**

The imidazoles have a broad spectrum of antifungal activity. In contrast to the polyenes, they are relatively resistant to light, hydrolysis and are soluble in organic substances.

The imidazoles have various mechanisms for their antifungal activity action. At low concentrations, miconazole, econazole, and ketoconazole affect the formation of ergosterol needed by the cell membranes. At higher concentrations, clotrimazole and miconazole can disrupt lysosomes, causing direct damage to cell membrane. Moreover, most imidazoles inhibit catalase and cytochrome C peroxidase intracellularly, causing accumulation of hydrogen peroxide and leading to cell death.

**Clotrimazole**

Clotrimazole has a wide spectrum of activity against numerous fungi excluding fusarium. It is used both topically and systemically. The topical preparation of clotrimazole is made by dilution in arachis oil to form a 1 percent solution (Table 4.2). It has been applied hourly for 48 to 72 hours and then tapered over 8 to 12 weeks. Orally, it is administered in a dosage range of 60 to 150 mg/kg/day. Clotrimazole is considered to be effective against Aspergillus keratitis. However, it is not given routinely due to its toxicity. Side effects of the systemic administration of clotrimazole may include anorexia, nausea, hallucinations, confusion, and gastritis. It should not be given in the first 3 months of pregnancy or to patients with a history of adrenal, or liver problems. Liver enzyme level elevations are seen normally with the use of clotrimazole, and these tend to return to normal once the drug is withdrawn.

**Miconazole**

Miconazole is a phenethylimidazole that is extremely stable in solution form. It has a broad spectrum of activity against Cryptococcus, Aspergillus, Curvularia, Candida, and Trichophyton.

For topical use, a 1 percent solution in arachis oil or a 10 mg/mL is used (Table 4.2). It is also available as a 2 percent dermatologic ointment.

<table>
<thead>
<tr>
<th><strong>TABLE 4.2</strong></th>
<th>Routes and dosage of antifungal agents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antifungal agent</strong></td>
<td><strong>Route</strong></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Topical (freshly prepared)</td>
</tr>
<tr>
<td>Natamycin</td>
<td>Topical suspension</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Topical</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Topical, Oral</td>
</tr>
<tr>
<td>Miconazole</td>
<td>Topical suspension, Subconjunctival</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Topical, Oral</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Topical, Oral</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>Topical, Oral</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Topical, Oral</td>
</tr>
</tbody>
</table>

Topical use of miconazole may cause surface toxicity after prolonged use.

**Ketoconazole**

Ketoconazole can be used topically as well as systemically.

A topical preparation may be formulated in a 1 to 5 percent concentration by dissolving in arachis oil (Table 4.2). Ketoconazole is primarily used for oral administration. It is well absorbed from the gastrointestina

**Triazoles**

The triazoles were developed in order to increase the spectrum of activity and reduce the side effects of their predecessors, the imidazoles.

**Fluconazole**

Fluconazole is now used mainly for the treatment of Candida keratitis. A topical 1 percent solution is
14 The 2 mg/L aqueous solution for intravenous use can also be applied topically to provide a higher concentration of the antifungal agent.

Oral fluconazole can be given in a dose of 50 to 40 mg/day, with the usual adult dose being 200 mg/day. Systemic side effects of fluconazole include gastritis, headaches, rash, hepatotoxicity, Stevens-Johnson syndrome, and thrombocytopenia.

**Itraconazole**

Itraconazole also has, like fluconazole, a wider spectrum of activity than the imidazoles in vitro. However, clinically it is found to be active against Candida species. The oral administration of itraconazole appears to have less penetration than other triazoles into the cornea and aqueous. Itraconazole is used in its oral preparation as an adult dose of 200 mg/day. Systemic side effects of itraconazole include gastrointestinal upset, hypertriglyceridemia, and hypokalemia.

**Voriconazole**

A new azole antifungal agent, Voriconazole, is derived from Fluconazole and exhibits a wider spectrum of activity against Candida, Aspergillus and Fusarium. It exerts its effect from inhibition of cytochrome P450-dependent 14 alpha sterol demethylase, an enzyme involved in the ergosterol biosynthetic pathway. The minimal inhibitory concentration of voriconazole (0.5 μg/ml) is less as compared to other imidazoles.

Topical variconazole 1% has to be prepared in pharmacy as it is not commercially available and is given in recalcitrant fungal keratitis if there is not response to topical natamycin and amphotericine B therapy.

### Antiviral Agents

**IDOXURIDINE**

Idoxuridine is a nucleoside analog of thymidine. It was the first topically effective antiviral drug produced (Table 4.3). However, due its need for frequent application, toxicity and availability of newer and better antiviral drugs, it is no longer used.

**VIDARABINE**

Vidarabine is a water insoluble antiviral agent. It acts by the inhibiting viral DNA synthesis. It can be administered topically in ointment form and systemically through the intravenous route (Table 4.3).

### TRIFLURIDINE

Trifluridine is a fluorinated nucleoside analog of thymidine. It is used topically in the form 1 percent solution. It is not used systemically as it rapidly degrades in the bloodstream and is not very selective in sparing the host DNA.

### ACYCLOVIR

Acyclovir is a synthetic purine nucleoside analog derived from guanine. It is a highly potent antiviral agent. Acyclovir interferes with DNA synthesis, thus inhibiting virus replication. It can be administered both topically and systemically. In the topical form it is used as a 3 percent ointment (Table 4.3).

Acyclovir has antiviral activity against HSV types 1 and 2 (HSV-1 and HSV-2), Varicella zoster virus, Epstein-barr virus and Cytomegalovirus.

Topical use of acyclovir ointment is well tolerated. No significant toxic effects have been seen in patients following topical treatment of herpetic ocular infections with 3 percent acyclovir ophthalmic ointment for 14 days. Systemic side effects of oral acyclovir include nausea, vomiting, and headache. Other adverse reactions include diarrhea, dizziness, anorexia, fatigue, edema, skin rash, leg pain, medication taste, and sore throat.

### VALACYCLOVIR

Valacyclovir is a prodrug of acyclovir and has been used in the treatment of herpes simplex keratitis. It is given orally in the dose of 1000 mg 3 times a day for 7 days.

---

**TABLE 4.3**

<table>
<thead>
<tr>
<th><strong>Antiviral Agents</strong></th>
<th><strong>Dosage and route of administration of antiviral agents</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Idoxuridine</td>
<td>Topical 0.1% solution and 0.5% ointment</td>
</tr>
<tr>
<td>Vidarabine</td>
<td>Topical ointment 3%</td>
</tr>
<tr>
<td>Trifluridine</td>
<td>Topical solution 10 mg/ml</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Topical ointment 3% Systemic 200-800 mg (oral capsules)</td>
</tr>
<tr>
<td>Valacyclovir</td>
<td>Systemic 1000 mg</td>
</tr>
</tbody>
</table>
The advantage of using this drug is that it has to be given less frequently.

**Adjuvants**

**CORTICOSTEROIDS**

Topical corticosteroids have a role to play in healing corneal ulcers as well as herpetic stromal viral keratitis. They decrease the inflammation and also the consequent corneal scarring.

**Topical Corticosteroids**

Topical preparations are available in the form of solutions, suspensions, or ointments. Phosphate and hydrochloride preparations are relatively hydrophilic and thus are water soluble. Acetate and alcohol derivatives are hydrophobic and fat soluble. Owing to the respective polarities, phosphates are generally formulated as solutions, whereas acetates are generally formulated as suspensions and ointments. Acetates, owing to their hydrophobic nature, appear to penetrate the cornea more than the phosphates.

Dexamethasone phosphate penetrates into the cornea and aqueous within 10 minutes. It reaches a peak within 30 to 60 minutes and remains there from several hours to 24 hours.

Likewise, one percent prednisolone phosphate is highly soluble with optimal corneal levels.

**Cycloplegics**

Infectious keratitis can induce a ciliary spasm which is partially responsible for the severe pain associated with most types of infectious keratitis. Topical cycloplegics help to alleviate this pain by relaxing the ciliary muscle.

**TYPES OF CYCLOPLEGICS**

**Atropine**

It is the strongest cycloplegic available and is used as either 1 percent solution or 1 percent ointment applied three times a day.

**Homatropine**

It is available in the form of a 2 percent solution and is administered 3 to 4 times per day.

**Anti-glaucoma Medications**

Intraocular pressure may rise in the acute phase of infectious keratitis.

**TOPICAL ROUTE**

For a mild to moderate rise in intraocular pressure topical therapy is instilled in the form of beta blockers like timolol maleate 0.5 percent.

**SYSTEMIC ROUTE**

Acetazolamide is used frequently to control intraocular pressure in cases of microbial keratitis. In case of very high intraocular pressure mannitol may be given.

**Acetazolamide**

It is a carbonic anhydrase inhibitor and is available in the form of oral tablets. It is given in the dose of 500 mg qid. The main side effects are nausea, gastritis and hypokalemia.

**Mannitol**

Mannitol is an osmotic agent which is available as a 20 percent solution and is given intravenously. It is not metabolized in the body. Its dosage is 1.5 to 2 g/kg body weight and it is given rapidly over 30 to 45 minutes. It can cause volume overload in the body. For this reason blood pressure should be monitored during its administration.

**References**


Work Up of Corneal Ulcer

SECTION 2
Introduction

History taking and clinical examination are crucial for the diagnosis and management of microbial keratitis. A meticulous history helps to identify predisposing incidents, risk factors and can provide clues to etiological diagnosis of microbial keratitis. The ophthalmologist must be able to distinguish microbial keratitis from other inflammatory diseases of the cornea. Similarly, a careful clinical examination with slit-lamp biomicroscopy may help in establishing the diagnosis.

Multiple factors may alter the clinical symptoms of keratitis. Antecedent partial antibiotic therapy, or combination antibiotic-corticosteroids therapy may mask or blunt the distinctive symptoms.

History and Symptoms

The classical symptoms of corneal ulceration include the presence of pain, watering, discharge, photophobia, decrease in visual acuity and swelling of lids.

PAIN

The occurrence of pain is a significant symptom of corneal ulcer. The severity of pain may range from minimal discomfort to an excruciating pain. The type of causative organism and the depth of the ulceration influence the severity of pain.

In general, superficial ulcers are more painful than deep corneal ulcers. This is due to the rich sensory nerve supply present in the superficial part of the cornea.

A small dendrite of Herpes simplex or a Candida species cause minimal pain and may only be associated with foreign body sensation. In contrast, a patient with Acanthamoeba keratitis presents with an excruciating pain, during corneal epithelial involvement due to radial keratoneuritis. The pain is usually out of proportion to the objective clinical findings, the alleviation of which may require strong analgesics and narcotics.

A sudden relief in pain, in a case of progressing corneal ulcer may be indicative of the perforation of the ulcer.

REDNESS AND PHOTOPHOBIA

Like pain, redness and photophobia are prominent symptoms in cases of corneal ulcer. The severity and duration of these symptoms may vary from case to case. An associated conjunctivitis may cause severe redness as in gonococcal, pneumococcal and Haemophilus infections.

DISCHARGE

Almost all cases of corneal ulcer present with a complaint of discharge from the affected eye. The type of the discharge may be watery, mucoid, mucopurulent or frankly purulent.

A watery discharge usually occurs due to a viral ulcer or a small bacterial ulcer or else it may also be due to reflex tearing. However, microbial keratitis caused by Pseudomonas and Gonococcus species is associated with a mucopurulent discharge (Fig. 5.1). Corneal ulcers caused due to Pseudomonas are particularly associated with a greenish-yellowish discharge (Fig. 5.2). A membranous discharge is seen with keratitis caused by Corynebacterium diphtheria.

DECREASED VISUAL ACUITY

Most cases of corneal ulcer report with history of sudden decrease in vision. The extent of decrease in vision depends on duration, severity and location of the lesion, involvement of other ocular structures and the success of concurrent therapy, if any.
Work Up of Corneal Ulcer

Central corneal ulcers, particularly those caused by organisms like *Pseudomonas* species, *Staphylococcus aureus*, and *Fusarium* species are invariably associated with significant loss of visual acuity. Other factors that can reduce visual acuity include the presence of an associated pupillary membrane, hypopyon, cataract, glaucoma and endophthalmitis.

The vision may not be severely reduced in cases which have small, peripheral ulcers, such as those caused by Herpes simplex and non-coagulase *Staphylococcus*.

In *Acanthamoeba* keratitis, vision may not be significantly reduced during the initial stages, when the corneal epithelium alone is involved. Later, as the infection spreads deeper into the corneal stroma, visual acuity is severely reduced.

Onset of Disease

The pattern of onset of microbial keratitis depends on the nature of predisposing factor/s, virulence of the offending organism and status of the ocular and systemic immunity of the patient.

Cases of bacterial keratitis usually report with a sudden onset and rapid progression of the corneal infection. This in fulminant cases may also cause a threat to vision loss and potential corneal perforation. If a patient presents with history of pain, photophobia, sudden diminution of vision and discharge of 1-2 days duration bacteria like *Staphylococcus aureus* (Fig. 5.3), *Pseudomonas aeruginosa* and *Pneumococcus* species are likely organisms responsible for the occurrence of corneal ulcer.

However, certain bacteria like *Moraxella*, coagulase negative *Staphylococcus*, *Nocardia* species and atypical *Mycobacteria* cause corneal ulcers that present with gradual onset and have an indolent course.

Microbial keratitis caused by fungi and parasites like *Acanthamoeba*, also have a chronic course. The course of development of *Acanthamoeba* keratitis is variable. It may emerge slowly over weeks or months or may rapidly worsen within several days after presumed inciting event. The course of infection is usually chronic and gradually progressive and may have some periods of temporary remission.

PREDISPOSING FACTORS

Identification of a predisposing factor is an integral component of history taking in a case of corneal ulcer and is essential for successful management of microbial keratitis.
Normally, corneal epithelium along with the tear film is a formidable barrier against the invasion of the microbes. However, in certain situations this barrier is compromised and organisms invade the corneal tissue and cause infection.

Various risk factors may make the cornea susceptible for developing microbial keratitis (Table 5.1). These risk factors may be ocular, systemic and occupational. In some cases more than one factor may be present or a co-existence of an ocular or systemic factor may be present.

**Ocular Factors**

The various ocular factors which may predispose to the occurrence of corneal ulcer include corneal trauma, prolonged use of topical corticosteroids, use of contact lenses, precorneal tear film disorders, eyelid disease, and ocular surface disease.¹

**TRAUMA**

Corneal injuries cause a breach in the intact corneal epithelial barrier which is the first line of defense against the occurrence of infectious keratitis.² Seemingly trivial trauma with contaminated matter, foreign bodies, makeup or contact lenses may also cause inoculation of the organism.³

<table>
<thead>
<tr>
<th>TABLE 5.1</th>
<th>Predisposing factors in case of corneal ulcer</th>
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<tbody>
<tr>
<td>• Ocular</td>
<td>Trauma</td>
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<tr>
<td></td>
<td>Contact lenses</td>
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<tr>
<td></td>
<td>Lid and adnexal infections</td>
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<tr>
<td></td>
<td>Ocular surface disease</td>
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<tr>
<td></td>
<td>Allergic eye disorders</td>
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<tr>
<td></td>
<td>Bullous keratopathy</td>
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<tr>
<td></td>
<td>Topical medications</td>
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<tr>
<td></td>
<td>Prior ocular surgery</td>
</tr>
<tr>
<td>• Systemic</td>
<td>Diabetes mellitus</td>
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<tr>
<td></td>
<td>Sjögren’s syndrome</td>
</tr>
<tr>
<td></td>
<td>Steven-Johnson syndrome</td>
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<tr>
<td></td>
<td>AIDS</td>
</tr>
<tr>
<td></td>
<td>Advanced malignancies</td>
</tr>
<tr>
<td></td>
<td>Connective tissue disorders</td>
</tr>
<tr>
<td></td>
<td>Alcoholics</td>
</tr>
<tr>
<td></td>
<td>Extremes of age</td>
</tr>
<tr>
<td></td>
<td>Measles malnutrition</td>
</tr>
<tr>
<td>• Occupational</td>
<td>Farmers</td>
</tr>
<tr>
<td></td>
<td>Animal handlers</td>
</tr>
<tr>
<td></td>
<td>Gardeners</td>
</tr>
</tbody>
</table>

Corneal trauma caused by vegetative matter is known to predispose to occurrence of mycotic (Fig. 5.4) and Acanthamoeba keratitis.⁴

If there is history of human or animal bites along with extensive necrosis of the tissue, anaerobic infections should be suspected.⁵

**CONTACT LENS**

The contact lens wear is one of the commonest predisposing factor for the occurrence of keratitis especially in series from western literature.⁶ The type of contact lens use, the duration of use of contact lenses and the method of lens care hygiene and disinfection and frequency of lens replacement should be enquired. Proper contact lens hygiene decreases the chances of microbial keratitis but infections have been reported to occur even when the patients were compliant with an adequate lens care hygiene.⁶

It has been demonstrated that the risk of contact lens induced keratitis is more with the extended wear soft contact lenses and overnight wear as compared to daily wear hard and soft contact lenses (Fig. 5.5).⁷,⁸ Infections with organisms such as Pseudomonas species have also been reported with orthokeratology.⁹

History of smoking coupled with contact lens wear is known to increase the risk of microbial keratitis as it increases the amount of corneal hypoxia.⁸ The daily disposable soft contact lenses in our experience have a lower risk of infectious keratitis compared with other lens wear regimens. However, reports have shown that at least some risk remains.¹⁰,¹¹
Work Up of Corneal Ulcer

Use of contaminated and non-sterile solutions such as homemade saline solution or tap water may be used to rinse, store or lubricate contact lenses which is typical of contact lens induced keratitis due to *Acanthamoeba*. *Acanthamoeba* keratitis may also occur due to contamination from swimming pool and muddy water (Fig. 5.6).

**LID AND ADNEXAL INFECTIONS**

Ulcerative keratitis caused by Pneumococcus has a known association with dacryocystitis. Likewise, infection with Actinomyces occurs more commonly in cases of canaliculitis. Any abnormality in the lid such as trichiasis, coloboma, ectropion, entropion, lagophthalmos, exophthalmos, proptosis, blepharitis and meibomitis may predispose the occurrence of infectious keratitis.

**OCULAR SURFACE DISEASES**

Conditions that affect the ocular surface causing a reduction of the integrity of and adherence of corneal epithelium such as dry eye syndrome, chemical injuries, allergic eye disease, can predispose to infection by opportunistic organisms (Fig. 5.7). All ocular surface disorders particularly the dry eye disturb precorneal tear film and increase the chances of adhesion of microorganisms to corneal epithelium.

**BULLOUS KERATOPATHY**

Bullous keratopathy caused by corneal endothelial decompensation disturbs the barrier function of the precorneal tear film and the corneal epithelium. The corneal surface becomes irregular and corneal erosions and epithelial defects develop. All these factors facilitate the entry of microbes in the corneal tissues. The risk factors for development of corneal infection in these cases include prolonged bullous keratopathy time, steroid use and use of bandage soft contact lens. Ulcerative keratitis developed in 4.7 percent of patients with bullous keratopathy in a study by Luchs et al.

**TOPICAL MEDICATIONS**

Amongst the topical medications, the prolonged use of topical corticosteroids is known to predispose the occurrence of infectious keratitis (Fig. 5.8). Topical
Clinical Examination

corticosteroids adversely affects the precorneal tear film and local ocular immunity, and can cause bacterial and fungal keratitis. Also, corticosteroids prevent neutrophil migration in response to chemotactic factors released during microbial keratitis. Moreover, there is impaired opsonization when encapsulated bacteria cause microbial keratitis. Corneal super-infection has been reported after indiscriminate use of corticosteroids in cases of Apollo conjunctivitis.

Continued use of non-steroidal anti-inflammatory agents, anesthetics, inappropriate use of antibiotics, antiviral and anti-glaucoma medications such as timolol may also predispose the occurrence of infectious corneal ulcer if used for a very long time. All these drugs cause toxic changes in the corneal epithelium.

The use of traditional eye medicines in developing countries has also been known to predispose the occurrence of keratitis (Fig. 5.9).

PRIOR OCULAR SURGERY

Ocular surgeries like cataract surgery, pterygium surgery, keratoplasty (Fig. 5.10) photorefractive keratectomy and laser in situ keratomileusis (LASIK) may be complicated by the occurrence of microbial keratitis (Fig. 5.11). Infectious keratitis particularly with Mycobacterium chelonei has been reported after LASIK surgery both in sporadic as well as in epidemic forms.

Systemic Factors

Certain systemic diseases like diabetes mellitus (Fig. 5.12), acquired immunodeficiency syndromes, Sjögren’s
syndrome and Steven-Johnson syndrome may act as risk factors for the occurrence of microbial keratitis. Moraxella lacunata ulcers are usually associated with alcoholics, diabetics and debilitated patients.\textsuperscript{27}

**Diabetes Mellitus**

The basement membrane and the hemidesmosome attachments to the corneal epithelium are impaired in the diabetes. Diabetics also have duplicated and thickened basement membrane and its anchoring fibrils to the stroma are weak. These changes in basement membrane may cause persistent corneal epithelial erosions predisposing it to the occurrence of infectious keratitis (Fig. 5.12).

**Acquired Immunodeficiency Syndromes and Advanced Malignancies**

An impaired immune status, as seen in acquired immune deficiency syndromes and advanced malignancy render the patient susceptible to developing microbial keratitis, especially by the unusual bacteria.\textsuperscript{26} These cases are prone to develop Candida keratitis. Further, the course of keratitis is more fulminant in these cases.

**Connective Tissue Diseases**

Systemic diseases like rheumatoid arthritis may adversely affect the healing of the corneal ulcer (Fig. 5.13). They may also predispose to corneal melting and predispose to secondary infectious keratitis.

**Other Systemic Factors**

*Pseudomonas* keratitis occurs more frequently in cases of burn patients, semicomatose or comatose patients, patients with corneal exposure and patients on prolonged ventilator support.\textsuperscript{9} In children, microbial keratitis may occur in association with conditions such as measles, diarrhea and malnutrition.\textsuperscript{28}

**Occupational Factors**

Some occupations may render a person susceptible to developing microbial keratitis. Mycotic keratitis occurs more commonly in persons involved in farming and agriculture and outdoor laborers.\textsuperscript{9} They are prone to be inflicted by minor vegetative or plant trauma while handling farms. It also occurs more commonly in conjunction with the use of gardening tools (e.g. nylon–line lawn trimmers) without protective eyewear. Certain type of keratitis such as due to *Listeria monocytogenes* is known to occurs in cases of animal handlers.\textsuperscript{8}

**Clinical Examination and Signs**

Examination of any patient of an ocular disease begins with the general examination of patient. A close look is made at the facial structures to look for the presence of any lesion like blisters of herpes zoster or simplex, presence of any facial palsy, abnormal blink rate and excessive corneal and conjunctival exposure.

The level of visual acuity and the size of the lesion are two important indicators of severity of a case of...
infectious keratitis. A particular type of organism may present with a characteristic type of clinical picture that may provide an important clue to etiological diagnosis. A patient of ulcerative keratitis should be examined as follows:

**Record of Visual Acuity**

It is usually possible to record only uncorrected visual acuity (UCVA) in an eye with ulcerative keratitis. A Snellens’ chart may be used to record the visual acuity. UCVA should be recorded at the time of presentation and also at subsequent follow-up visits. Monitoring of the visual acuity is an important parameter to check the severity and the progress of the ulcer. In young children an attempt should be made to record the visual acuity using Teller’s acuity cards.

**External Ocular Examination**

Eyelids and lacrimal sac area should be examined for any abnormality besides recording usual concomitant presence of lid swelling and circumciliary congestion.

**EYELIDS**

Eyelids should be examined for the presence of any abnormality such as trichiasis, coloboma, entropion, lid lag, ectropion, lagophthalmos, proptosis, exophthalmos and blepharitis. These conditions act as predisposing factors and may require simultaneous management along with that for infectious keratitis.

If there is a history of foreign body going in to the eye, the eyelids should be everted and the fornices should be examined. If required and possible a double eversion of upper eyelid should be done to find and remove the foreign bodies.

**LACRIMAL SAC**

A gross external examination of lacrimal sac area should undertaken to rule out dacryocystitis. Pneumococcal corneal ulcers may be associated with lacrimal sac infections. A sac pressure test or regurgitation test is performed by pressing over the lacrimal sac area just medial to the medial canthus and observing regurgitation of discharge from the puncta. A positive regurgitation test indicates the presence of dacryocystitis.

**Slit-lamp Biomicroscopy**

A detailed slit-lamp biomicroscopic examination is mandatory to examine a case of infectious keratitis. Slit-lamp biomicroscopy should include an examination of the precorneal tear film, conjunctiva, cornea, anterior chamber, iris, lens and anterior vitreous.²⁹

**CONJUNCTIVA**

The bulbar conjunctiva and the upper and lower tarsal conjunctiva should be examined for the presence of any lesion or diseases like vernal catarrh and atopic conjunctivitis. Conjunctival reaction is usually not specific but may occasionally help in diagnosis. An associated severe conjunctivitis is present in gonococcal, pneumococcal and Haemophilus infections usually with chemosis and sometimes with conjunctival pseudomembranes. Presence of chemosis or membranes should thus be recorded.

Bacterial infections may be characterized by the presence of papillae which are tufts of capillaries infiltrated by inflammatory cells and separated by septa bound down by tarsus and are suggestive of inflammation of the conjunctiva. Prominent limbal vessels or circumciliary flush is usually seen in bacterial keratitis.

Presence of discharge may also be seen and a note of its color and consistency should be made. A watery discharge is generally seen with viral keratitis, whereas a mucoid or mucopurulent discharge is associated more often with bacterial keratitis. The color of the discharge may also help in clinching the final diagnosis, as a greenish purulent discharge is seen in cases of *Pseudomonas* keratitis (Figs 5.2 and 5.14).

**PRECORNEAL TEAR FILM**

In a case of active keratitis the precorneal tear film and the meniscus typically consist of numerous cells and debris unlike an inactive corneal ulcer. A breach in the corneal epithelium is more characteristically seen in cases of active keratitis unlike a non-infective infiltrate where the overlying epithelium is intact. However, in some cases of deep ulceration, the overlying epithelium may be completely intact.

One should also look for the presence of filaments in the precorneal tear film to rule out the presence of filamentary keratitis. Fluorescein dye may be used to stain the filaments.

**CORNEA**

The examination of the cornea includes the examination of the corneal ulcer as well as the examination of the surrounding cornea.
Work Up of Corneal Ulcer

Corneal Ulcer

The location, size and depth of the corneal ulcer, the dimensions of the epithelial defect and the infiltrate and the status of the surrounding cornea and underlying endothelium should be recorded in great detail using the slit-lamp biomicroscopy (Fig. 5.15). Documentation of the size and features of ulcer are done by clinical photography and using color-coded diagrams. Apart from this if there is any scleral involvement, it should be recorded.

On examination under the slit-lamp the following features of the corneal ulcer should be recorded:

- **LOCATION OF CORNEAL ULCER**

  The location of the ulcer should be carefully marked on a schematic diagram of the cornea. Some organisms have a predilection for certain specific locations on the cornea. The ulcer may be central (Fig. 5.9), paracentral, peripheral (within 3 mm of limbus) or total. In some of the cases half or whole of the cornea may be involved. Shield ulcers associated with vernal keratoconjunctivitis usually have lower border in the upper half of the visual axis (Figs 5.16A and B).

  Central ulcers cause a severe reduction in visual acuity and carry a poor visual prognosis even after

Figure 5.14: *Pseudomonas keratitis*

Figure 5.15: Slit-lamp biomicroscopy of corneal ulcer

Figure 5.16A: Giant papillary conjunctivitis in a case of vernal conjunctivitis

Figure 5.16B: Shield corneal ulcer in same case in Figure 5.16A
resolution as compared to the peripheral ulcers. Central ulcers are usually caused by Staphylococcus species (Fig. 5.17). *Mycobacterium tuberculosis* and herpes simplex virus may cause peripheral corneal ulcers.

**SHAPE OF THE ULCER**

Bacterial corneal ulcer usually appear as punched out lesions (Fig. 5.17), where as fungal ulcers are usually dry looking and may have hyphate or feathery margins (Fig. 5.18). Infectious keratitis caused by herpes simplex may have classically dendritic pattern or appear to have amoeboid or geographic patterns (Figs 5.19A and B). Corneal ulcers due to Acanthamoeba may have a ring shaped lesion (Fig. 5.6).

**MARGINS OF ULCER**

The margins or the edges of the ulcer may vary according to the stage of the ulcer. For instance, a healing infectious ulcer and sterile corneal ulcers have well-defined margins (Fig. 5.20A) whereas an active corneal ulcer has indistinct margins (Fig. 5.20B). Mooren’s ulcer have a classical overhanging margins (Fig. 5.21).

**SIZE OF CORNEAL ULCER**

Micrometer present in a standard slit-lamp should be used to record the size of corneal ulcer at the initial presentation and all follow-up visits till the resolution of ulcer. In a case of ulcerative keratitis measurement of
Work Up of Corneal Ulcer

The size of the lesion is an important parameter for monitoring the success of the treatment modality.

A grading system of corneal ulcers may be used to objectively study and monitor the progress of a case of infectious keratitis\(^\text{30}\) (Table 5.2).

**TABLE 5.2**
Grading of corneal ulcer

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of ulcer (mm)</td>
<td>&lt; 2</td>
<td>2-5</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>Depth of ulcer (%)</td>
<td>&lt; 20</td>
<td>20-50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Infiltrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>— Density</td>
<td>Dense</td>
<td>Dense</td>
<td>Dense</td>
</tr>
<tr>
<td>— Extent</td>
<td>Superficial</td>
<td>Extension up to mid-stroma</td>
<td>Deeper than mid stroma</td>
</tr>
<tr>
<td>Scleral involvement</td>
<td>Not involved</td>
<td>Not involved</td>
<td>May be involved</td>
</tr>
</tbody>
</table>


The epithelial defects should be stained with fluorescein dye and the size should be measured with the help of slit-lamp micrometer at all follow-up visits (Fig. 5.22B). The staining of the lesion may be achieved either by putting a drop of the dye from the dropper or applying dye stained strips in the conjunctival sac. Most corneal specialists prefer to use fluorescein or rose bengal strips to stain the lesion. These strips are more useful as they are sterile as compared to the drop of the dye solutions. Because of the fluorescence property of the fluorescein dye, the stained epithelial defect is examined using the cobalt blue light of the slit-lamp. The minute

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**Figure 5.20A**: Active corneal ulcer with hazy margins

**Figure 5.20B**: Healing corneal ulcer with distinct margins

**Figure 5.21**: Mooren’s corneal ulcer with over hanging margins
Clinical Examination

Figure 5.22A: Corneal ulcer with epithelial defect and infiltration

Figure 5.22B: Florescein staining shows size of epithelial defect is smaller than size of infiltrate

Figure 5.23: Multiple infiltrates

details of the fluorescein in staining pattern may be lost within 2-3 minutes; therefore, it is imperative that the lesion is examined soon after putting the dye in the conjunctival sac. Characteristic corneal staining patterns may be seen in different types of infectious keratitis (Figs 5.19B and 5.22A).

Rose bengal dye may be used to stain the margins of the epithelial defects particularly in cases of herpetic keratitis the margins of which stain with the dye as these are loaded with virus particles.

Apart from the size of the epithelial defect, any associated epithelial edema, if present, should also be noted and graded.

INFECTION

Infiltration is an intrinsic component of suppurative infectious keratitis. The infiltrates may be single or multiple and may be of varying sizes depending on the organism involved and the duration of the infection (Figs 5.22A and 5.23). The infiltrate should be measured in the two largest dimensions and recorded on a schematic cornea diagram. If there are multiple infiltrates as seen in some fungal corneal ulcers, or cases of polymicrobial keratitis, each one of them is measured separately at all visits (Fig. 5.23). An estimated depth of the stromal ulceration may be determined by comparing the adjacent uninvolved corneal thickness. It should be recorded at every follow-up visit.

The ulcers may be graded as mild, moderate or severe depending on the size of the ulcer, depth of ulcer, infiltrate depth and density and involvement of sclera.

Corneal Sensations

Corneal sensations should be assessed with the help of a cotton wisp or esthesiometer, if available. In cases of herpetic keratitis the corneal sensations are markedly decreased.

Special Characteristics

Some organisms produce lesions of particular shapes, color or have some distinctive features. A mere clinical
examination of such lesions may aid in establishing the etiological diagnosis, although clinical observation should not replace the laboratory investigation of direct microscopy and culture of corneal scrapings.2

The classical examples of such features are dendritic keratitis, radial neurokeratitis, satellite lesions, hyphate extensions, black/brown discoloration, and black/brown discoloration. The lesions clinical characteristics and the possible etiological diagnosis are highlighted in Table 5.3.

**TABLE 5.3**
Characteristics of various corneal ulcers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic keratitis</td>
<td>Herpes simplex, Herpes zoster</td>
</tr>
<tr>
<td>Radial neurokeratitis</td>
<td>Acanthamoeba</td>
</tr>
<tr>
<td>Amoeboid ulcer</td>
<td>Herpes simplex</td>
</tr>
<tr>
<td>Black/brown discoloration</td>
<td>Dematious fungal keratitis, corneal ulcer in a tattooed corneal opacity</td>
</tr>
<tr>
<td>Yellow-greenish discharge</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Satellite lesions</td>
<td>Fungus, Acanthamoeba</td>
</tr>
<tr>
<td>Ring shaped stromal infiltrates</td>
<td>Acanthamoeba, fungus</td>
</tr>
<tr>
<td>Disk shaped corneal edema</td>
<td>Herpes simplex</td>
</tr>
<tr>
<td>Limbal peripheral lesions</td>
<td>Staphylococcus aureus, Herpes simplex, immunological and connective tissue disorders, Mooren's ulcer</td>
</tr>
<tr>
<td>Endothelial plaques</td>
<td>Fungus</td>
</tr>
<tr>
<td>Feathery hyphate edges</td>
<td>Fungus</td>
</tr>
<tr>
<td>Dry surface</td>
<td>Fungus</td>
</tr>
<tr>
<td>Crystalline infiltrates</td>
<td>Streptococcus viridans</td>
</tr>
<tr>
<td>Overhanging margins</td>
<td>Mooren's ulcer</td>
</tr>
</tbody>
</table>

**Surrounding Cornea**

The cornea surrounding the lesion may be, clear or hazy due to edema depending on the virulence of the organisms. Gram-positive cocci and Candida tend to cause localized lesions with distinct borders and minimal surrounding edema. Some organisms like Pseudomonas produce ulcers in which the surrounding cornea becomes hazy and grossly edematous appearing like a ground glass. Clearing of surrounding corneal edema after the initiation of medical therapy is an early sign of resolution of the ulcer.

**Corneal Vascularization**

Superficial or deep corneal vascularization of varying extent may be seen in cases of infectious keratitis. A quadrant wise record of corneal vascularization should be made. Presence of corneal vascularization suggests the commencement of the healing of the corneal ulcer (Figs 5.24A to C).

**CORNEAL THINNING/PERFORATION**

The ulcer should be closely monitored for the development of corneal thinning, descemetocele and perforation. In the presence of shallow anterior chamber and low intraocular pressure a Seidel’s test should be performed in all cases.32

When the escape of aqueous is suspected, fluorescien dye is applied directly to the site of the leakage. When present, escaping aqueous dilutes the fluorescien to flow down the surface of the eye. The application of concentrated fluorescien results in a dark non-fluorescing background against which the diluted and now brightly fluorescing dye is highly visible, even in the presence of modest flow.

Occurrence of corneal perforation is an indication of failure of medical therapy (Figs 5.25A and B). Severe corneal thinning and corneal perforation warrant immediate surgical therapy in the form of application of glue or a tectonic patch graft.
The documentation of the corneal ulcer may be done using color clinical photographs or using detailed schematic drawings.

**Color Photographs**

In all cases of corneal ulcer a colored photograph of the diffuse as well as the slit section of the corner should be taken. Measurements should be made which includes the diameter of the ulcer in maximum diameter and the meridian perpendicular to it. Apart from it the maximum diameter of the infiltrate is measured along with its diameter in the meridian perpendicular it. Linear measurements are made by focusing the slit-beam and adjusting the slit length to match the distance measured and recording the micrometer reading in the frontal sketch. Alternatively calibrated reticules may also be placed in the slit-lamp microscope ocular to obtain the measurements.

**Schematic Drawings**

In places where clinical photography is not available, drawings of the corneal ulcer along with the surrounding cornea should be made using a standard scheme of color-coding and shading. Drawings are less expensive than photographs, provide immediate record, present total picture at a glance and highlight details which may be difficult to photograph. This allows the ophthalmologist to follow the course of the disease. It also provides a standard form of notation, which assures better communication where more than one ophthalmologist follows-up the patient.

Black color is used to outline the corneal limbus and to indicate scars resulting from keratitis degeneration and foreign bodies. Thus a scar resulting from bacterial keratitis, calcific band keratopathy and lipid keratopathy are denoted in black.

Blue designates edema, using shading for diffuse stromal edema, small circles for epithelial edema or lakes of fluid within the stroma, and wavy lines to depict the Descemet’s membrane.

The contours of the cornea are drawn in black both in frontal and cross-sectional views. In the frontal picture the level of the cross-sectional view is indicated by an arrow. The cross-sectional view is drawn at 3 levels—first at epithelium, other at stroma and the last at the level of endothelium.

Brown indicates melanin or iron pigmentation including pupil and iris.

Red is used to depict blood vessels and rose bengal staining. Red wavy lines indicate subepithelial vessels, straight lines indicate stromal vessels and dotted lines indicate ghost vessels. The wavy lines of superficial vessels begin outside the limbus circle, whereas straight lines of stromal vessels begin at the margin of the circle. Solid shading depicts hemorrhage and red dots indicate area stained by rose bengal.

Orange denotes inflammation and indicates presence of white blood cells, which may be in the following forms—stromal infiltrate, hypopyon or keratic...
precipitates (The use of orange/yellow color for opacities such as lipid degeneration and spheroidal degeneration should be avoided).

Green indicates fluorescien staining of the cornea and colored dots represent punctuate epithelial keratopathy, small lines depict filaments and shaded outline demonstrate epithelial defects. Green color is also used to depict the location of lens and the vitreous.

**ANTERIOR CHAMBER**

The anterior chamber reaction may range from mild flare and cells to severe hypopyon formation. A record of hypopyon and its characteristics should be made using a slit-lamp biomicroscopy (Fig. 5.26). The size of the hypopyon should be measured using the slit-lamp micrometer.

Fixed immobile hypopyon is a feature of fungal keratitis. In order to test for the mobility of the hypopyon, following a slit lamp examination, the patient is asked to lie supine for 10 minutes and a slit-lamp evaluation is then done. In cases of the fixed hypopyon, there is no change in position of the hypopyon as demonstrated by the height of the hypopyon which is similar. However, in cases of mobile hypopyon, the position, i.e. the upper level or the height of the hypopyon decreases and there is actual movement (Figs 5.27A and B).

**IRIS**

Uveal inflammation is a common occurrence with infectious keratitis. Iris involvement may occur in the

form of synechiae. Presence of rubeosis iridis should also be noted.

If the ulcer perforates uveal prolapse may occur and this may later form a corneoiridic scar.

**PUPIL AND LENS**

Any abnormality in the pupil size, its shape and location should be recorded using the slit-lamp biomicroscopy. If visible, lens should be examined for the presence of cataract or any other abnormality.

**Scleral Involvement**

Any involvement of the sclera should be recorded as this helps in prognosticating the case as well as change
Clinical Examination

in the management protocol, since this warrants the use of systemic antimicrobial agents. Sclerokeratitis usually occurs in cases of immunologic disorders and Acanthamoeba keratitis.

POSTERIOR SEGMENT

Usually, it is not possible to view the vitreous and retina in a case of corneal ulcer due to the presence of hazy cornea. However, if the ulcer is small and peripheral, slit-lamp biomicroscopy may be done to visualize the anterior one-third of the vitreous. Additionally, an indirect ophthalmoscopy may be performed to check for the involvement of the posterior segment (Fig. 5.28).

INTRAOCULAR PRESSURE

Digital tonometry in the experienced hands is the most practical method of assessing intraocular pressure in cases of corneal ulcer in our experience. Secondary glaucoma may be present in some cases and should be appropriately treated.

Ultrasoundography

In cases where it is not possible to view the posterior segment, an ultrasoundography A scan and B scan may be undertaken to assess the posterior segment. A clear posterior segment evaluation rules out the presence of vitritis/endophthalmitis. In cases of perforated corneal ulcers, endophthalmitis, choroidal and retinal detachment should be excluded. This helps to prognosticate the cases, especially if a therapeutic keratoplasty is contemplated in these cases.

Varying Clinical Picture

Based on the presenting clinical history, antecedent risk factors, predisposing ocular and systemic factors and distinctive clinical signs, infectious keratitis may be easy to diagnose. However, there may be following factors, which may alter the typical clinical features of infectious keratitis:

1. Previously partially treated corneal ulcers with antibiotic therapy alone or combination antibiotic-corticosteroid therapy may mask or blunt the distinctive/classical features of corneal ulcer. Withdrawal of the previous topical corticosteroids may cause an initial resurgence of the stromal inflammation and necrosis if they are withdrawn abruptly, and hence during the initial management this can confuse the initial response to treatment.

2. In cases of partially treated ulcers especially with topical antibiotics, the normal conjunctival flora is suppressed and this leads to invasion with saprophytic bacteria so that the typical features of infection are masked.

3. Additionally, the toxicity of certain topical drugs such as aminoglycosides, anesthetics, idoxuridine and amphotericin B may simulate corneal infection by causing epithelial and stromal ulceration and multiple focal suppurative inflammation.

4. Non-infectious keratitis may mimic suppurative keratitis especially, if the inflammation is marked.

5. The clinical features may be different in a patient with previously compromised corneas. Individuals with neurotrophic or exposure keratopathy may develop ulceration, which may be indistinguishable from infectious keratitis.

References


Investigations

Ocular and systemic investigations including microbiological investigations are an integral part of work-up of a case of infectious keratitis. These investigations help in making accurate etiological diagnosis and initiation of appropriate therapy.

**Systemic Investigations**

Systemic investigation should be done in cases of sterile corneal ulcers.

Systemic investigations in cases of peripheral ulcerative keratitis include laboratory tests, which should focus on the systemic diseases (Table 6.1). This include a hemogram in cases of immuno compromised individuals and blood sugar examination in suspected cases of diabetics.

A complete blood count including ESR, urine analysis, blood urea, nitrogen and creatinine. IgM-rheumatoid factor, which is positive in cases of Rheumatoid arthritis, scleroderma, polyarteritis nodosa, Wegner’s granulomatosis, systemic lupus erythematosus, sarcoidosis should also be sent for. Circulating antibodies such as ANA (>90%), anti-DNA (70%) are positive in systemic lupus erythematosis. Angiotensin converting enzyme (ACE) is elevated in sarcoidosis and antineutrophil cytoplasmic antibodies (ANCA) are present in 96 percent cases of Wegner’s granulomatosis. Hepatitis B surface antigen is sent for in suspected cases of polyarteritis nodosa and fluorescent treponemal antibody absorption (FTA-ABS) in suspected cases of syphilis.

**Ocular Investigations**

The ocular investigations include the clinical investigations and the microbiological investigations.

**Clinical Investigations**

The clinical investigations include the estimation of the intraocular pressure and posterior segment evaluation by ultrasonography to rule out any evidence of concomitant endophthalmitis.

**INTRAOCULAR PRESSURE**

Tonopen can be used to measure intraocular pressure in cases of non-perforated corneal ulcers. Digital estimation of the intraocular pressure may also be done to rule out secondary glaucoma. The intraocular pressure will be low in cases of perforated corneal ulcers.

**ULTRASONOGRAPHY**

Ultrasonography B scan should be done to evaluate the status of the posterior segment in cases of corneal ulcers where endophthalmitis is suspected.

**Microbiological Investigations**

Laboratory procedures for the diagnosis of keratitis are directed towards the detection of bacteria, fungi or parasites. A well-equipped dedicated ocular microbiology laboratory staffed with trained technicians who are specially oriented towards handling ocular specimens, have a greater leverage over a general microbiology laboratory. The samples should be collected and a smear examination should be done. Further, the scrapings should also be directly inoculated into the culture media.
and sensitivity of the organisms to the antibiotics should be obtained.

COLLECTION OF SAMPLES
The samples should be collected at the initial presentation prior to the start of anti-microbial therapy. The treatment can be initiated based on the results of smear examination and, if required modified in accordance with the culture and sensitivity results.

Various types of samples may be collected to aid the diagnosis of corneal ulcer (Table 6.2). The most important sample for microbiological examination is the corneal scraping1,2 (Fig. 6.2). The samples should also be obtained from the contact lenses, contact lens case and contact lens solutions if the patient is a contact lens wearer. If there is any lacrimal sac discharge, it should also be sent for microbiological investigations. Although it has also been recommended to obtain samples such as a conjunctival swab and an eyelid swab, it has not proved to be of much help in our experience.

Corneal Scraping
Corneal scraping is the most valuable specimen in cases of corneal ulcer and its examination is the main stay in the diagnosis and subsequent management (Fig. 6.1).

Anesthesia
Corneal scraping is performed under topical anesthesia preferably after instillation of two drops of 0.5 percent proparacaine in the lower fornix of the affected eye. Topical 0.5 percent proparacaine is least bactericidal as compared to other anesthetic agents such as tetracaine and xylocaine.1,3 Proparacaine provides adequate anesthesia within one minute and does not cause intense stinging on first installation.

General anesthesia and sedation may be required in children, uncooperative adults or mentally impaired patients.3, 4

Instruments
Corneal scraping is obtained using a Kimura’s spatula (Fig. 6.3). The other instruments for corneal scraping, are 26-gauge needle, Bard Parker blade #57 (Becton

<table>
<thead>
<tr>
<th>TABLE 6.2</th>
<th>Samples for diagnosis of corneal ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyelid swab- Not of much use</td>
<td></td>
</tr>
<tr>
<td>Conjunctival swab - Not of much use</td>
<td></td>
</tr>
<tr>
<td>Corneal scraping- Most important</td>
<td></td>
</tr>
<tr>
<td>Contact lens, contact lens case and solution- Must in contact lens wear</td>
<td></td>
</tr>
<tr>
<td>AC paracentesis (Hypopyon)- Deep ulceration or when insufficient material is present</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.1: Corneal scraping

Figure 6.2: Fixation of smear by heating

Figure 6.3: Kimura’s spatula
Dickinson, Franklin Lakes, New Jersey), hypodermic needle, surgical blade no 15 and calcium alginate swab. The platinum spatula has been traditionally used for corneal scraping. It is rapidly sterilized with a Bunsen burner and cools rapidly between scrapings. A modified platinum spatula is also available with a rounded flexible tip, which is modified with a honing stone to create a narrow tapered roughened edge to enhance removal of corneal material.3

We routinely use a 23-gauge needle to scrap the ulcer. It is easier to get good quantity of corneal scrapings due to the sharpness of this instrument. However extreme caution is need in cases of dry and deep ulcers especially thin corneas.

**Technique**

A lid speculum is applied gently to separate the lids. Care should be taken not to rub the cornea against the superior flange especially while applying the speculum.

The scraping may be done under a slit lamp or under an operating microscope. Under direct illumination, the ulcer is inspected. Any mucous or debris on and around the ulcer is carefully cleaned with a sterile swab stick. Then, using a Kimura Spatula or a Bard Parker Knife or a 23-gauge needle, the leading edges and base of the ulcer are scraped. *Streptococci pneumoniae* is more readily found at the edge of the ulcer whereas *Moraxella* is more likely to be present at the ulcer base.1 Since the material obtained from corneal scraping may not be adequate, it should be directly inoculated into the culture media rather than placing it first into the transport media.4 Care should be taken to ensure that the instrument is moved in one direction only. Multiple scrapings must be obtained to enhance the yield of the organisms. One should be careful not to touch the eyelids or the lashes while collecting the sample to avoid contamination.

More recently, calcium alginate swabs moistened with trypticase soy broth provides another method of collecting corneal specimens.6 Studies have demonstrated higher yield of bacteria⁷ as well as fungi⁸ when this method was used as compared to the platinum spatulas.

**Difficulties in Collection of Corneal Scrapings**

There may be various situations where it is difficult to obtain the samples for corneal scraping.⁹

In cases of small corneal ulcers, which are less severe, and in cases of non-suppurative cases of keratitis there may be insufficient material to inoculate.

In advanced keratitis with severe keratolysis and descemetocele, it may not be possible to obtain multiple scrapings.

In some cases the overlying epithelium may be intact and it may be required to disrupt the corneal epithelium using a surgical blade. In cases of deep stromal keratitis, microsurgical scissors, a no. 11 Bard Parker blade or a small trephine may be used to obtain an adequate sample.

**EXAMINATION**

Smears are prepared by scraping the ulcer and gently transferring the material on the spatula on to the glass slide over an area of approximately 1 cm in diameter. An etched or wax pencil mark on the slide obviates the need to search for the area smeared under the microscope. At least two slides are prepared, one for Gram staining, and the second for KOH wet preparation. An additional smear may be prepared for special stains such as Giemsa, periodic-acid-Schiff, calcofluor or Gomori modified methenamine silver stain for which gelatin coated slide is preferred. ⁹ KOH wet mount preparation is undertaken to identify the fungal organisms. Gram staining of the corneal smears is done in all cases routinely to identify the organisms (Table 6.3). Special stains may be needed only in certain circumstances (Table 6.3).

In order to use the smear to guide the anti-microbial therapy, an adequate material should be there and careful staining techniques should be followed. Prior use of anti-microbial agents, an insufficient sample, excessive heat fixation, mechanical damage to the cell wall, poor staining techniques and failure to examine the whole slide may give erroneous results.⁴

**Routine Smears and Stains**

**Potassium Hydro-oxide Wet Mount Preparation**

The scraped material is spread out as thinly as possible with the help of spatula on the slide. One drop of 10 percent KOH solution is put on the scrapings and a slide cover is placed. The slide is examined under a microscope. The KOH helps in loosening the corneal stromal lamellae and exposing more fungal filaments (Fig. 6.4A). It also stains the filaments in a very light yellow color. 10 percent KOH mount examined by conventional microscope is a useful test in helping identification of fungi and *Acanthamoeba*. The test has high sensitivity (92%) and a high specificity (96%), can
Overall, Gram’s stain is accurate in 61 percent of cases of bacterial keratitis. 4 If performed correctly, Gram’s stain identifies the organism correctly in up to 75 percent of the cases caused by a single organism and in 37 percent cases of polymicrobial keratitis. 12

Difficulties in Gram Staining

Sometimes only small number of organisms is present in the smear, which may be difficult to identify as these are usually present in areas which contain necrotic epithelial cells and numerous large polymorphonuclear cells.

Gram-negative organisms are more difficult to identify than the Gram-positive organism due to their
lighter color. Gram-negative organism may appear gram-positive if decolorization is inadequate. Caution is also mandatory against various artifacts, which may accompany Gram’s stain such as stained deposits, carbon particles, talcum powder, sodium chloride, crystals, melanin and granules. Precipitated gentian violet may mimic gram-positive cocci. If the Gram stain reagents are not used frequently, yeast may grow in the solutions and periodic filtering helps to remove these particles.

### TABLE 6.3
**Gram Stain Procedure**

- **Fix smear** by either of the following methods:
  - Place in methanol for 5-10 min and allow to air dry: preferred method
  - Pass the slide, through flame two or three times. Allow cooling.
- Flood the slide with **crystal violet stain.** Allow stain to remain on for 1 minute
- Rinse gently with tap water.
- Flood slide with **Gram’s iodine solution.** Allow solution to remain on for 1 minute.
- Rinse gently with tap water.
- Decolorize with **decolorizer** solution until the color stops running from the smear
- Rinse gently with tap water.
- Flood the slide with **Safranin stain.** Allow stain to remain on for 30 seconds.
- Rinse gently with tap water.
- Allow to air dry.

### TABLE 6.4
**Gram stain morphology of organisms**

<table>
<thead>
<tr>
<th>Type of stain</th>
<th>Organism visualized</th>
<th>Color of the organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram stain</strong></td>
<td>Bacteria</td>
<td>Gram positive-purple</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram negative-pink</td>
</tr>
<tr>
<td><strong>Acridine orange</strong></td>
<td>Bacteria</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td></td>
<td>Fungi Acanthamoeba</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td><strong>Calcofluor white</strong></td>
<td>Fungi Acanthamoeba</td>
<td>Bright green</td>
</tr>
<tr>
<td></td>
<td>cysts Acanthamoeba</td>
<td>Bright green</td>
</tr>
<tr>
<td></td>
<td>trophozoites Acanthamoeba</td>
<td>Reddish orange</td>
</tr>
<tr>
<td></td>
<td>Mycobacteria</td>
<td></td>
</tr>
<tr>
<td><strong>Acid fast</strong></td>
<td>Mycobacteria</td>
<td></td>
</tr>
</tbody>
</table>

### Special Stains

#### Giemsa Staining

The Giemsa stain is usually used to determine the type of inflammatory cells present. We do not recommend its use routinely. This stain differentiates bacteria from fungi, and also identifies chlamydia inclusion bodies and cysts and trophozoites of *Acanthamoeba* species. It identifies the normal and inflammatory cells. With Giemsa technique the bacteria appear dark blue in color. The yeast cells and fungal hyphae absorb the stain and appear purple or blue while the cell walls and the septations do not stain. The conventional Giemsa stain takes 60 minutes to perform, although a rapid 15-minute modification of the stain is also available.

#### Ziehl-Neelsen Acid-fast Stain

Special stains include the use of carbol-fuchsin or Ziehl-Neelsen acid-fast stain for identification of suspected *Mycobacteria, Actinomyces* or *Nocardia*. *Mycobacteria* are acid fast (Fig. 6.4D), *Nocardia* stain variably, whereas *Actinomyces* are non-acid fast.

The use of this stain is based on the resistance of the mycobacterial species and certain strains of *Nocardia* to decolorization by strong mineral acids after staining with basic carbol fuchsin. This resistance is due to the presence of intact cell walls that contain specific lipid unsaponifiable wax fraction.

#### Calcofluor White

Calcofluor white binds to chitin and cellulose. Because the cell walls of the yeast and filamentous fungi are composed of chitin and cellulose, these organisms stain bright green with calcofluor white under epiflorescent microscope (Fig. 6.5). Blankophone stain, which is similar to calcofluor can also be used to identify fungal hyphae (Fig. 6.6). The cysts of *Acanthamoeba* likewise also have chitin and cellulose and also stain bright green. The trophozoites of *Acanthamoeba* stain reddish–orange in color.

#### Acridine Orange

The acridine orange is a chemoflorescent dye, which stains fungi and bacteria yellow-orange against a green background when the pH is acidic and an epiflorescent microscope is used to visualize these organisms. It identifies gram-positive and gram-negative bacteria, yeast and hyphal forms of fungi and both the
Work Up of Corneal Ulcer

trophozoite and cyst form of *Acanthamoeba*. The Gram’s stain can be directly done on a slide where prior staining with acridine-orange has been done without destaining. The acridine orange stains accurately predicts culture results in 71 to 84 percent of cases in comparison to 62 to 79 percent for Gram’s stain.16,17

**Modified Grocott-Gomori Methenamine Silver Nitrate Stain**

For fungal infections, this stain is more reliable than the Gram, Giemsa, or KOH stain. The specimens should be spread onto gelatin-coated slides. With the methenamine-silver nitrate stain, fungus cell walls and septa black and can be easily seen against the background, which is a faint transparent green.

**CULTURE EXAMINATION**

Culture on the standard media is the gold standard for the diagnosis of microbial keratitis.4 The culture media used to culture the organisms should be stored in a dedicated refrigerator and should be re-stocked frequently to avoid any contamination. In order to enhance the recovery of the organisms, the media should be warmed to the room temperature to prevent lethal cold shock to the organism.1

The corneal scrapings are routinely inoculated onto blood agar plate (Fig. 6.7), chocolate agar plate, Sabouraud’s dextrose agar (if fungus is suspected) (Fig. 6.8) and anaerobic media (if anaerobes are suspected) (Table 6.5). The selective media agar plates are inoculated by streaking the platinum spatula lightly over
the surface to produce a row of separate inoculation marks in a C shaped configuration. Fresh material should be obtained for each row of C streaks (Fig. 6.7). C streak method of inoculation differentiates valid growth from contamination as growth on the C streak is considered significant whereas outside this is a possible contamination.

Liquid thioglycolate broth is inoculated by transferring the material to cotton tipped applicator which has been moistened with trypticase soy broth or calcium alginate swab (Fig. 6.9). The swab is inserted to the bottom of the tube to enhance the growth of anaerobic organisms.

**Blood Agar**

Enriched media such as blood and chocolate agar help to isolate the fastidious organisms. Blood agar is the standard medium for the isolation of aerobic bacteria at 35°C and helps to support the growth of most saprophytic fungi at room temperature (Fig. 6.7). The agar is derived from the seaweed and produces optimal surface moisture, and addition of 5 to 10 percent red blood cells provides nutrients and an index of hemolysis. Rabbit and horse serum are preferred for supporting the growth of *Haemophilus*.

**Chocolate Agar**

Chocolate agar is prepared by the heat denaturation of blood and provides hemin (X factor) and diphosphopyridine nucleotide (V factor) essential for the growth of *Haemophilus*. It should be incubated at 35°C with 10 percent carbon dioxide. It also supports the growth of *Neisseria* and *Moraxella*.

**Sabouraud’s Agar**

Sabouraud’s agar consists of glucose and peptone agar. Yeast extract is added to improve nutritional characteristics and an antibiotic such as gentamicin or chloram-

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**TABLE 6.5**

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Growth</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean casein digest broth (trypticase soy broth)</td>
<td>Saturation of swabs</td>
<td>35°C</td>
</tr>
<tr>
<td>Blood Agar plate</td>
<td>Aerobic bacteria Facultative anaerobic bacteria Fungi</td>
<td>35°C</td>
</tr>
<tr>
<td>Chocolate Agar plate</td>
<td>Aerobic bacteria Facultative anaerobic bacteria <em>Neisseria</em> <em>Haemophilus</em> <em>Moraxella</em></td>
<td>35°C</td>
</tr>
<tr>
<td>Thioglycolate broth</td>
<td>Aerobic bacteria Anaerobic bacteria</td>
<td>35°C</td>
</tr>
<tr>
<td>Sabouraud’s dextrose agar plate with antibiotic</td>
<td>Fungi Nocardi</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Brain Heart infusion broth plate with antibiotic</td>
<td>Fungi Nocardi</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Special</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked meat broth</td>
<td>Anaerobic bacteria</td>
<td>35°C</td>
</tr>
<tr>
<td>Schaedler agar</td>
<td>Anaerobic bacteria</td>
<td>35°C</td>
</tr>
<tr>
<td>Thayer Martin Blood agar plate</td>
<td><em>Neisseria</em></td>
<td>35°C</td>
</tr>
<tr>
<td>Brucella blood agar plate</td>
<td></td>
<td>35°C</td>
</tr>
<tr>
<td>Lowenstein-Jensen media</td>
<td><em>Mycobacteria species</em></td>
<td>35°C with 3 to 10% CO₂</td>
</tr>
<tr>
<td>Middlebrook-Cohn agar</td>
<td><em>Mycobacteria Nocardia</em></td>
<td>35°C with 3 to 10% CO₂</td>
</tr>
</tbody>
</table>
phenicol is added to inhibit bacterial contamination (Fig. 6.8). The Sabouraud’s agar should not contain any additives such as cycloheximide as this inhibits saprophytic fungi commonly responsible for ocular infections.

Following primary cultures the fungi are transferred to the sporulating media for subculture of the species. Sabouraud’s agar plates are preferred to the slants because of the ease of inoculation, observation of colony growth, transfer to secondary media and dilution of inhibitory substances to the fungi.\(^1\)

**Thioglycolate Broth**

It promotes the growth of aerobic bacteria, as well as obligate and facultatively anaerobic organisms.\(^1\)

Thioglycolate broth grows aerobic and anaerobic bacteria at 35°C (Fig. 6.9). It consists of the basic nutrients required to support the growth of aerobic bacteria and also has sulf-hydryl compound that act as an oxygen-reducing agent to facilitate the recovery of the anaerobic bacteria. It also supports a number of saprophytic fungi.

The limitation of this media for the growth of anaerobic organisms is its inability to restrict the growth of the aerobic organisms.

**Pre-reduced Anaerobically Sterilized Media (PRAS)**

This is an ideal medium for the isolation of the anaerobic bacteria. A PRAS brucella blood agar plate enriched with vitamin K and hemin allows the growth of the anaerobes within 4 to 7 days. This is also recommended for isolation of nutritional variant streptococci, which may be a causative organism for infectious crystalline keratopathy.\(^1\) The anaerobic media should not be exposed to air and should be incubated in an anaerobic system such as anaerobic jar, anaerobic bag system or anaerobic chamber. In the GasPack Pouch system (Bacton Dickinson, Cockeysville) a packet that has sodium borohydride, sodium bicarbonate and citric acid generate hydrogen and carbon dioxide after water is added in order to create an anaerobic environment.

**Brain Heart Infusion (BHI)**

Brain Heart Infusion (BHI) broth at room temperature enhances the growth of filamentous fungi and yeasts. It is especially relevant in ocular infections as the inoculum material is small and it provides a more even exposure to the essential nutrients.

**Thayer-Martin Media**

This is an enriched chocolate media that suppresses the growth of the inhibitory components and selectively allows the growth of the *N. gonorrhoeae*.

**Lowenstein Jensen Media**

In cases of suspected atypical microorganisms, the cultures should also be sent on Lowenstein Jensen media. This medium allows the growth of Mycobacteria. It contains glycerol and egg mixture which provides fatty acids and proteins (Fig. 6.10).
**Micro-Antimicrobial Removal Device (Micro-ARD)**

In cases, which have been treated with anti-biotics previously, antimicrobial removal device can be used to increase the yield of the positive cultures. The commercially available blood ARD is modified for smaller ophthalmological samples by transferring 2.5 ml aliquots of all components to test tubes. The ARD is composed of sterile resins (amberlite XAD4 and C-249) which binds antibiotics suspended in sodium chloride. In a study by Osato et al the use of micro-ARD increased the isolation of organisms from 88 to 100 percent.²⁰

**Duration of Isolation of Organism**

Most aerobic bacteria responsible for keratitis are seen on standard culture media within 48 hours. In some cases the pathogen may be recognized in 12 to 15 hours. All plates should be examined daily with the help of dissecting microscope and liquid media should be evaluated for the presence of turbidity. In cases of severe keratitis the media should be evaluated after 12 to 18 hours of inoculation.

Growth outside the C streak should be disregarded as it implies contamination and circled with wax pencil. Indigenous organisms in the tear film may appear on the inoculation marks but may be distinguished on the basis of their sparse growth and isolation of the same organism from the ipsilateral lids or the conjunctiva, if these specimens have been taken.

Aerobic cultures of the corneal specimens should be held for 7 days, anaerobic cultures for 7 to 14 days and Mycobacterial and fungal cultures for 4 to 6 weeks before being reported as no growth.

**Interpretation of Culture Results**

The interpretation of the culture results should be made with regard to the clinical situation, the adequacy of the sample and the possibility of contamination by organisms present on the skin, eyelids and conjunctiva.

**Positive Culture**

Reported culture positive rates in presumed infectious keratitis varies from 40 to 73 percent.²¹,²²

Criteria for a significant positive culture by some investigators include: clinical signs of infection plus isolation of bacteria (10 or more colonies) on one solid medium and one additional medium, or isolation of fungi (any detectable growth) on any two media or one medium in the presence of a positive smear.²³ Although liquid media provide a highly sensitive method for demonstrating a pathogen, a positive culture from broth is less specific than a positive culture from solid media as it is difficult to quantify the broth cultures. Anaerobic bacteria are suspected in following situations: Pleomorphic, slender or fusiform morphology seen on Gram stain of corneal smear or culture, growth in the anaerobic zone of liquid medium or within the depth of solid agar, production of gas on liquid media and failure to grow organism in aerobic media despite organism detection in Gram stain.²¹

**Negative Cultures**

Negative cultures may be present truly in cases of sterile/non-infectious ulcers or due to prior partial antibiotic treatment, inadequate sampling methods, improper selection of the media and incubation conditions and false interpretation of the data.³ Negative cultures have been reported in 44 percent of cases in a series of 663 cases of microbial keratitis.²²

When the culture results are negative, antibiotic treatment can be suspended temporarily for 24 hours and rescraping is done following which repeat cultures are sent and examined.

**Mixed Organisms/Polymicrobial keratitis**

Polymicrobial keratitis is a distinct clinical entity²³. More than one organism in corneal cultures may be identified in 6 to 32 percent of cases depending on the laboratory techniques and the criteria for positive or negative culture.²²,²³ The most frequent combination in mixed microbial infections is an aerobic gram-positive coccus and a gram-negative rod. Rarely it may be a combination of bacteria and fungi.¹

As soon as the growth is detected, an estimate of the number of the colonies should be made, with a description of the colony morphology. Determination of the antibiotic sensitivities is done. Minimal inhibitory and bactericidal concentrations are usually available within a few days.
ANTIMICROBIAL SUSCEPTIBILITY TESTING

The preferred methods for testing the susceptibility of the antimicrobial agents are the standard disk diffusion method and the micro-dilution techniques (Fig. 6.11). The limitation of the ocular antimicrobial susceptibility testing is that the results of agar disk diffusion tests relate to the levels of drug in the serum rather than the concentration of the antibiotics achieved in the ocular tissues and fluids.

The quantitative minimal inhibitory concentration (MIC) estimation by the broth microdilution methods provide greater information about the ocular infections. Once a MIC value of a particular antibiotic is found out the bactericidal effect of the antibiotic may be titrated by sub culturing the clear broth on the antibiotic free zone.

Minimal Bactericidal concentration (MBC) is the concentration of the antibiotic, which reduces the growth of the bacterial strain by 99.9 percent. The minimum antibacterial concentration (MAC) is the inhibitory effect of the antibacterial agent, which is observed in 5.5 hours in which 90 percent of the bacterial population is inhibited.4 The post antibiotic effect (PAE) is the result observed after the bacterial population is exposed to antibiotics for approximately one hour followed by removal of the antibiotic either after enzymatic treatment or dilution and then subculturing to see the amount of bacterial population that remains.4

SEROLOGICAL INVESTIGATIONS

A variety of DNA probe assays are available for the confirmation of the results of the culture and for direct detection of the organisms. The expensive molecular microbiologic tests however, should not replace the time-tested culture and staining techniques, the efficacy of which have a proven track record. Such serological test may be divided into three categories:

i. Target amplification systems such as polymerase chain reaction (PCR), cell sustaining sequence replication (3 SR) or strand displacement amplification (SDA)

ii. Probe amplification systems which includes ligase chain reaction (LCR)

iii. Signal amplification in which the signal generated from each probe is increased by using compound probes or branched chain technology

These techniques detect whether DNA and RNA from a particular organism is present but do not detect the viability of the organism.

The advantages of PCR include greater speed than culture methods (up to 4 hours) and the ability to analyze specimens far from where they are collected. The cost of PCR to diagnose infections generally exceeds that of conventional culture methods, a factor that currently limits its widespread use.24 16S rDNA typing has been used as a rapid alternative to culture for identifying pathogens in patients with bacterial keratitis,25 whereas 18S ribosome gene is used to detect fungal keratitis.24

CONFOCAL MICROSCOPY

It is particularly useful when relatively large infecting organisms (15 microns) are present, as are seen in Acanthamoeba, filamentous fungal, microsporidial, and, possibly, Lyme Borrelia keratitis.25,26 Confocal microscopy can clearly demonstrate both the cyst and often the trophozoite forms of acanthamoeba in suspected keratitis. It also shows the enlarged corneal nerves accompanying radial neurokeratitis and the characteristic honeycomb-pattern intrastromal microcavities seen during the late stages of the disease.25

Retina Tomograph II—Rostock Cornea Module (HRTII-RCM) has also demonstrated the presence of fungi. Filamentous fungi-infected patient’s corneas reveal numerous high-contrast lines 200–300 μm in length and 3–5 μm in width, with branches at 90° angles (in cases of Fusarium) or 45° at angle (Aspergillus species).26

Candida albicans—infected patient’s cornea reveal numerous high-contrast elongated particles measuring
10–40 μm in length and 5–10 μm in width in the anterior stroma resembling Candida pseudo-filaments.

**CORNEAL BIOPSY**

Sometimes repeated smear examinations and cultures of corneal scrapings done by standard method do not demonstrate presence of any microorganisms. This may be true for certain cases of deep mycotic keratitis and intrastromal abscesses. A vertical or oblique incision can allow sampling using a sterile needle or a mini-spatula. Alternatively, a deep lamellar excision may be undertaken to reach a focal abscess.

In such cases, a diagnostic superficial keratectomy or corneal biopsy may be necessary to obtain microbe-infested tissue to make an accurate microbiological diagnosis. Corneal biopsy is superior to scraping for isolating fungus from a case of mycotic keratitis. The procedure is performed under topical anesthesia under an operating microscope. A dermatologic 2-3 mm trephine or a small Elliot microtrephine is advanced in to the anterior corneal stroma, to incorporate both the infected and the clinically normal 1 mm rim. Care is taken to avoid the visual axis as far as possible. A crescent blade or Bard Parker knife is then used to undermine the tissue, which may then be cut with a surgical blade or a microscissors and the tissue is excised (Fig. 6.12). The biopsy tissue is excised with a fine tooth forceps taking care not to crush the tissue. The specimen thus obtained should be divided in to pieces and subjected to smear examination, cultures and histopathological examination.

### References


Work Up of Corneal Ulcer

Types of Microbial Keratitis

SECTION

3
Bacteria are the most important cause of infectious keratitis. Prompt recognition, expedient evaluation and rapid initiation of antibiotic therapy are of vital importance. Most cases respond to medical therapy and yield gratifying results. With an advanced infection, devastating complications such as corneal thinning, corneal perforation scleral extension and consequently, endophthalmitis may occur.

There are no pathognomonic clinical signs that confirm a definite bacterial etiology. However, based on distinctive corneal signs such as the status of the epithelium, type of stromal inflammation and the site of the inflammation, a possible suspicion of the bacterial etiology may be reached which is confirmed on the laboratory examination.

**Risk Factors**

The eye is continuously exposed to a large number of bacteria, which form a part of ocular flora. However, only a small number of these bacteria cause keratitis. There are various pre-disposing factors, which may precipitate bacterial keratitis which include the following:

**CORNEAL TRAUMA**

Corneal trauma causing a breach in the intact epithelium along with concurrent inoculation of organisms can occur from vegetable matter, industrial foreign bodies, contact lenses, cosmetic application and administration of ocular medications causing bacterial keratitis\(^1\)\(^3\) (Fig. 7.1).

**EYELID DISEASE**

Ocular adnexal disorders such as blepharitis, dacryocystitis, ectropion with exposure, entropion (Fig. 7.2) with trichiasis or lagophthalmos (Fig. 7.3) lead to disturbed precorneal tear film that can predispose to bacterial keratitis.

*Figure 7.1: Keratitis in a case of repaired corneal perforation*

*Figure 7.2: Keratitis in a case of entropion*
OCULAR SURFACE DISORDER

Ocular surface disorders such as dry eye, Stevens Johnson syndrome and ocular burns can cause chronic corneal decompensation may predispose the eye to bacterial keratitis. Ocular surface disorders causes the disturbance of the tear film dynamics and leads to persistent epithelial defects (Fig. 7.4) which are secondarily infected and cause stromal ulceration.1

TEAR FILM DYSFUNCTION

Tear film dysfunction which predispose to bacterial keratitis include dry eye due to aqueous layer insufficiency, mucin layer abnormality due to goblet cell loss/dysfunction, lipid layer instability or lacrimal drainage obstruction.

PREVIOUSLY COMPROMISED LOCAL/ SYSTEMIC DEFENSE MECHANISMS

Topical corticosteroids, antifungal medications, contaminated ocular medications and anesthetics impair the immune mechanisms. The use of topical corticosteroids causes localized immune suppression by preventing neutrophil migration in response to chemotactic factors released as a consequence of microbial keratitis (Fig. 7.5).

Patients with diabetes are also more prone to bacterial keratitis.4 Patients with acquired immunodeficiency syndrome (AIDS) from infection of human immunodeficiency virus (HIV) do not appear to be at increased risk for bacterial corneal ulcers, but when they develop bacterial keratitis it takes a more fulminant course and ulcers are most often caused by *Pseudomonas*.5

CONTACT LENS USE

The contact lens keratitis may occur due to the contact lens per se, which compromises the corneal health as it causes corneal hypoxia (Fig. 7.5). Contact lenses cause hypoxia and increased corneal temperature.5-8 Extended soft contact lens wearers are at 10 to 15 times increased risk for bacterial keratitis as compared to daily lens wearers.7 Aphakic contact lens wearers have 6 to 9 times increased risk for bacterial keratitis.
greater rate of infectious corneal ulceration as compared to cosmetic wearers due to longer wear intervals, reduced defense mechanisms of a postoperative cornea or increased corneal hypoxia. Pseudomonas is the most common organisms causing contact lens-related ulcers, followed by Staphylococcus sp. and Serratia marcescens.

Further, the contact lens used may also be infected and infection may also occur as a consequence of improper care of the contact lenses or the contact lens case and the contact lens solution.

**Etiologic Organisms**

Although virtually any bacteria may cause keratitis, four principal groups of bacteria are primarily responsible. These include – the Micrococcaceae, Streptococcaceae, Pseudomonas and the Enterobacteriaceae(Table 7.1). Eighty seven percent of the bacterial keratitis has been attributed to these organisms.

Bacteria may be divided into two groups based on the structure of the cell wall. Gram-positive organisms, possess a thick pepti-doglycan layer, which resists physical forces. Gram-negative organisms, on the other hand, have a comparatively thicker peptidoglycan layer adjacent to the cytoplasmic membrane and a cell wall, which has large amounts of lipoprotein and lipopolysaccharide.

Mycobacteria have cell walls made of long chain fatty acids (mycolic acids), which resist decolorization by strong organic solvents and hence are acid fast.

Some bacteria may produce endospores, which are refractile bodies within the vegetative bacterial cell, so that the bacteria survive in adverse environment conditions of heat and dryness.

**REGIONAL DIFFERENCES**

The bacterial organisms most commonly identified show regional differences based on the prevalence, the type of pre-existing corneal pathology, the climate and the nature of referrals.

The predominant causative organism of bacterial keratitis in most studies are gram-positive organisms. Staphylococcus species is the most common cause of bacterial keratitis in most parts of the world in previously normal corneas as well as the already compromised corneas. Staphylococcus aureus is common in north and north-eastern United States, New York, Southern California, Florida, Canada and London.

Pseudomonas aeruginosa has been identified more frequently in southern United States, Hong Kong and Ghana.

<table>
<thead>
<tr>
<th>TABLE 7.1 Classification of bacterial organisms causing microbial keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic bacteria</strong></td>
</tr>
<tr>
<td><strong>Gram-positive cocci</strong></td>
</tr>
<tr>
<td>Micrococcaceae</td>
</tr>
<tr>
<td>– Staphylococcus aureus</td>
</tr>
<tr>
<td>– Staphylococcus epidermidis</td>
</tr>
<tr>
<td>Streptococcaceae</td>
</tr>
<tr>
<td>– Streptococcus pneumoniae</td>
</tr>
<tr>
<td>– A and B hemolytic streptococci</td>
</tr>
<tr>
<td>– Aerococcus</td>
</tr>
<tr>
<td>– Enterococcus</td>
</tr>
<tr>
<td><strong>Gram-positive bacilli</strong></td>
</tr>
<tr>
<td>Bacillaceae</td>
</tr>
<tr>
<td>– Bacillus cereus</td>
</tr>
<tr>
<td>– Bacillus subtilis</td>
</tr>
<tr>
<td>– Corynebacteria diphtheriae</td>
</tr>
<tr>
<td>– Corynebacteria xerosis</td>
</tr>
<tr>
<td>– Listeria monocytogenes</td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
</tr>
<tr>
<td>Pseudomonaceae</td>
</tr>
<tr>
<td>– Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Acinetobacter</td>
</tr>
<tr>
<td>Azoto bacter</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>– Klebsiella</td>
</tr>
<tr>
<td>– Serratia</td>
</tr>
<tr>
<td>– Proteus</td>
</tr>
<tr>
<td>– Citro bacter</td>
</tr>
<tr>
<td>– Enterobacter</td>
</tr>
<tr>
<td>– Escherichia</td>
</tr>
<tr>
<td><strong>Gram-negative diplococci</strong></td>
</tr>
<tr>
<td>Neisseria</td>
</tr>
<tr>
<td><strong>Gram-negative diplobacilli</strong></td>
</tr>
<tr>
<td>Moraxella</td>
</tr>
<tr>
<td><strong>Gram-negative cocacobacilli</strong></td>
</tr>
<tr>
<td>– Hemophilus</td>
</tr>
<tr>
<td><strong>Gram-positive filaments</strong></td>
</tr>
<tr>
<td>– Mycobacterium</td>
</tr>
<tr>
<td>(Non-tuberculosis)</td>
</tr>
<tr>
<td>– Nocardia</td>
</tr>
<tr>
<td><strong>Anaerobic bacteria</strong></td>
</tr>
<tr>
<td><strong>Gram-positive cocci</strong></td>
</tr>
<tr>
<td>Peptococcaceae</td>
</tr>
<tr>
<td>– Peptococcus</td>
</tr>
<tr>
<td>– Peptostreptococcus</td>
</tr>
<tr>
<td><strong>Gram-positive bacilli</strong></td>
</tr>
<tr>
<td>Propionibacterium acne</td>
</tr>
<tr>
<td>– Actinomycetes</td>
</tr>
<tr>
<td>– Clostridium</td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
</tr>
<tr>
<td>Fusobacterium</td>
</tr>
<tr>
<td>– Bacteroides</td>
</tr>
<tr>
<td>– Capnocytophaga</td>
</tr>
<tr>
<td><strong>Gram-negative dipslococci</strong></td>
</tr>
<tr>
<td>Veillonella</td>
</tr>
<tr>
<td><strong>Spirochaetales</strong></td>
</tr>
<tr>
<td>– Treponema</td>
</tr>
<tr>
<td>– Borrelia</td>
</tr>
<tr>
<td>– Leptospira</td>
</tr>
</tbody>
</table>

In India the organisms, which have been identified most commonly, include Staphylococcus epidermidis and Streptococcus species.

**SPECIFIC ORGANISMS**

**Staphylococcal Organisms**

Staphylococcal species are the most frequently isolated bacterial organisms from corneal ulcers. The most susceptible corneas to these infectious include those with
Types of Microbial Keratitis

Corynebacterium diphtheriae

*Corynebacterium diphtheriae* may penetrate intact corneal epithelium due to its toxin. *Corynebacterium xerosis* is frequently associated with *Moraxella* infections.

**Actinomycetes**

Actinomycetes and related organisms such as *Nocardiopsis* resemble bacteria more than fungi. Actinomycetes are gram positive, branching filamentous fungi, which may rarely cause keratitis. *Nocardiopsis* organisms are ubiquitous in soil and cause keratitis especially after trauma.

**Spore Forming Anaerobic Bacteria**

Spore forming anaerobic bacteria such as *Clostridia* may rarely cause keratitis especially after contamination with soil and may be associated with gas bubbles in the corneal tissue or anterior chamber.

**Nonspore-forming Anaerobic Organisms**

Nonspore-forming anaerobic organisms should be suspected as a cause of keratitis following human or animal bites. They are associated with extensive necrosis of the tissue, gas formation in tissue or foul discharge. The most frequent anaerobes are *Peptostreptococcus*, *Peptococcus*, *Propionibacterium*, *Bacteroides* and *Fusobacterium* species. *Propionibacterium* species are non-spore forming anaerobic bacteria and can cause keratitis in healthy and compromised cornea.

**Non-tuberculous Mycobacteria**

Recently, non-tuberculous mycobacteria, including *Mycobacterium fortuitum*, *M. chelonae*, *M. gordonae* and *M. avium intracellular* are emerging as causes of indolent keratitis especially after surgical procedures such as laser-in-situ keratomileusis. Out of all the species of Mycobacteria, *Mycobacterium chelonae* is most frequency isolated. They may also cause keratitis after injury with a foreign body. *Mycobacterium leprae* may also cause keratitis with involvement of the peripheral corneal nerves.

**Less Common Organisms**

Organisms which are associated less commonly with keratitis include *Enterobacteriaceae* (*Serratia*, *Proteus*, *Azotobacter* and *Neisseria* species, *Serratia marcescens* has been associated with keratitis in contact lens wearer and
contaminated eye drops. *Listeria monocytogenes* causes severe suppurative keratitis in animal handlers and farmers. Syphilitic as well as non-syphilitic spirochaetal infection can cause non-suppurative stromal keratitis.

**Clinical Features**

**GENERAL SYMPTOMS**

The general symptoms of bacterial keratitis include decreased visual acuity, photophobia, pain, redness and discharge. The severity of symptoms depends on the organisms, the condition of the host and the duration of the symptoms before the patient is examined.

**GENERAL SIGNS**

An infectious corneal ulcer should be distinguished from a non-infectious corneal ulcer by the following features: The precorneal tear film and meniscus in an actively infected ulcer are typically seen via slit lamp examination to contain numerous cells and debris, which are absent in a non-infected corneal ulcer. The corneal epithelium is absent over areas of active infection (unlike a non-infected ulcer) and the stromal inflammation is suppurative.

Associated conjunctivitis is present with *Gonococcal, Pneumococcal* and *Haemophilus* infections. They may frequently present with chemosis and sometimes conjunctival pseudomembrane also. Papillae, which are tufts of capillaries infiltrated by inflammatory cells and separated by septa bound down by tarsus, usually accompany bacterial infections and are indicative of conjunctival inflammation.

Discharge varies from copious amount of clear fluid to a greenish-yellow purulent material.

**SPECIFIC FEATURES**

Bacteria, which can invade an intact cornea, include *Neisseria, Corynebacterium, Haemophilus, Shigella* and *Listeria*.

**Gram-positive Organisms**

*Staphylococcal Ulcers*

*Staphylococcus aureus* is present in 15 percent of cultures obtained from lids of normal individuals and non-coagulase *Staphylococcus* is present in 85 percent of cultures obtained from lids of normal individuals. *Staphylococcal* ulcers occur in compromised corneas such as in patients with bullous keratopathy, chronic herpetic keratitis, dry eyes, rosacea keratitis or atopic disease.

The ulcer tend to remain localized with distinct borders and non-edematous surrounding stroma (Fig. 7.6). Long standing *Staphylococcal* ulcers tend to infiltrate deeper areas and may cause intrastromal abscess (Fig. 7.6) and may lead to corneal perforation. Occasionally, multiple stromal micro infiltrate satellites may be seen resembling fungal infections. They are generally associated with mild to moderate anterior chamber reaction.

*Pneumococcal Ulcers*

They generally occur following trauma to the cornea and cannot invade a healthy cornea directly. They are particularly associated with the cases of dacryocystitis. Infiltration starts at the site of injury and rapidly spreads towards the center of cornea. Severe anterior chamber reaction associated with hypopyon is usually present. Fibrin deposition may be seen on endothelial side of the ulcer and a deep stromal abscess may form, with intervening stroma relatively clear. If untreated corneal perforation and melting may occur rapidly (Fig. 7.7).

*Alpha Hemolytic Streptococci*

They are responsible for infectious crystalline kerato-pathy which has been discussed in details in chapter.

**Gram-negative Organisms**

*Pseudomonas ulcers*

*Pseudomonas* keratitis presents as a rapidly evolving infection that may lead to perforation and loss of eye, if left untreated. Occasionally they may also be slowly progressive or have an indolent course.

Infection results when a traumatized cornea is exposed to the organism. Following adhesion of the organisms to the damaged epithelium and stroma, invasion into stroma occurs within an hour. The infection begins with an epithelial defect, superficial edema and micro-infiltration of stroma, which occurs as early as 6 to 8 hours after injury. The infiltration extends peripherally and deeply and within 18 to 24 hours it is extensive. The ulcer spreads symmetrically
Types of Microbial Keratitis

and concentrically to involve whole width and depth of cornea.

A characteristic feature of Pseudomonas ulcer is diffuse epithelial graying which characteristically occurs away from the main site of epithelial and stromal infiltration. A ring ulcer is often seen at 48 to 96 hours with an untreated infection. The progressive untreated ulcer is associated with melting of the cornea (Figs 7.8A and B), and with greenish-yellow mucopurulent discharge which is adherent to the ulcer. This leads to descemetocle formation and eventual perforation within 2 to 5 days of onset of infection.

Other Gram-negative Rods

Other gram-negative bacilli include Klebsiella, Escherichia coli and Proteus, which most commonly cause indolent ulcerations in previously compromised corneas with milder anterior chamber reaction.

Moraxella Ulcers

The ulcer caused due to Moraxella is typically indolent with only mild to moderate anterior chamber reaction. It is usually oval and located in the inferior part of the cornea. It tends to remain localized as it spreads into
Bacterial Keratitis

the deep stroma. Some ulcers may have prolonged, moderately severe stromal and anterior chamber reactions with endothelial decompensation despite proper treatment.

Anaerobes

Anaerobic infections usually follow corneal injuries with contaminated soil.21

Clostridium species are suggested by the presence of gas bubbles, which are visualized in the anterior chamber in the corneal stroma or under the epithelium.

Listeria monocytogenes causes ulcerative keratitis in animal handlers.1 Propionibacterium acnes and Peptostreptococcus are rare anaerobes. Actinomyces infections are associated with canaliculitis.1

Nocardia species inhabit the soil and cause indolent ulceration and are introduced into the eye following trauma.24 These ulcers simulate fungal corneal ulcers and occasionally have elevated, hyphate edges and often produce satellite lesions and cracked-windshield appearance of the cornea.

Mycobacterium fortuitum inhabits the soil and cause indolent ulcers, which progress slowly over weeks.22,23 The bed of the ulcer has cracked windshield appearance with minimal changes in the surrounding cornea or the anterior chamber.

Microbiologic Work Up

A provisional diagnosis can be reached after the clinical work up of a case which is confirmed on microbiology. Traditionally, the treatment regimen should be started only after smears and cultures have been taken. Corneal scraping from the ulcer area is obtained and is sent for microbiologic work up which includes direct microscopy and culture.

The smears include the Gram’s smear, Giemsa staining, KOH wet mount preparation and Grocott-Gomori methenamine-silver staining, especially if fungal infection is suspected. Acid-fast stains are necessary when M. fortuitum, Actinomyces sp. or Nocardia sp. are suspected as in cases of indolent corneal ulceration.

The material obtained should also be inoculated directly on the culture media, rather than placed on carrier or transport media, since specimen obtained usually contain very few organisms. The specimens are C streaked on Blood agar plate, Chocolate agar,

Sabouraoud agar plate or blood agar plate at room temperature (Fig. 7.9) for fungi and chopped meat glucose broth or Thioglycolate medium with hemin and vitamin K for anaerobes.

In case of contact lens keratitis, contact lens, case and solution should also be sent for microbiologic examination.24

However, sometimes the Gram stain may fail to reveal the offending organisms, in that case, it is inadvisable to delay treatment while awaiting the results of culture. The empirical treatment with the broad-spectrum antibiotic drops should thus be started while waiting for the culture reports.

Treatment

Ideally a patient of bacterial keratitis should be hospitalized especially if the compliance of the patient is in doubt or it is a more severe form of disease. Reliable patients with mild or moderate ulcers may be treated as outpatients with careful follow-up. In general the most rapidly destructive microbial keratitis is bacterial and should be treated as bacterial corneal ulcer until a definitive diagnosis is made.

Until the results of the definitive cultures are available, Gram’s stain is a quick and helpful tool for initiating a rational antibiotic therapy. If done properly,
Gram’s stain may identify pathogen in up to 75 percent of the cases caused by single organism and 37 percent of the cases caused by mixed organisms. We do routine Gram’s staining and KOH wet mount preparation in all cases of suspected microbial non-herpetic keratitis.

The initial management of cases of bacterial keratitis includes the use of medical therapy. However, in cases of corneal perforation surgical modalities may be resorted to.

**MEDICAL THERAPY**

The frequent administration of the broad-spectrum antibiotic topical drops is the mainstay of the treatment. Additional supportive therapy includes the use of topical cycloplegic agents, antiglaucoma medications and use of lubricants.

**Choice of Antibiotics**

The treatment with topical antibiotics is initiated with either a combination fortified therapy or monotherapy.

**Types of Antibiotic Therapy**

In cases where no treatment has been given earlier it is mandatory to take corneal scrapings and send them for culture examination.

In case where topical antibiotics were given before one can suspend the topical medications for 12 to 24 hours before obtaining the specimen for corneal scraping.

**Combination Therapy**

We prefer to use the combination therapy in cases of bacterial keratitis. A combination therapy consists of a cephalosporin, which acts against the gram-positive cocci and some of the gram-negative rods and an aminoglycoside which acts against the gram negative organisms. Alternatively one of newer generation fluoroquinolones may also be combined with fortified cefazolin. The newer generation fluoroquinolones cover some gram-positive organisms and most of the significant gram-negative rods including many *Pseudomonas* species.

Combined fortified 5 percent cefazolin sodium and 1.3 percent tobramycin sulphate are given in hourly dosage for the initial 48 hours (Table 7.2). Following an initial response to this therapy, the frequency of the drugs is tapered and the topical drugs are given two hourly dosage. Some people prefer to use 10% cefazolin sodium instead of 5% cefazolin sodium in the combination therapy. The cephalosporin provides coverage against gram-positive cocci and some of the gram-negative rods while the aminoglycoside are covers most of gram-negative bacilli.

Following clinical response the frequency of the antibiotics is reduced 4 hourly after 72 hours and is subsequently tapered over the next few days.

**Monotherapy**

Ciprofloxacin 0.3 percent, ofloxacin 0.3 percent, Gatifloxacin 0.3 percent or moxifloxacin 0.5 percent can be given as monotherapy and is effective against most corneal pathogens. Additionally they are also effective against most strains of aminoglycoside resistant *Pseudomonas*, methicillin resistant *Staphylococcus* and

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**TABLE 7.2**

<table>
<thead>
<tr>
<th>Preparation of fortified topical antibiotics*</th>
</tr>
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<tbody>
<tr>
<td>1. <strong>Cefazolin</strong> 50 mg/mL or cefazidime 50 mg/mL</td>
</tr>
<tr>
<td>a. Add 9.2 mL of artificial tears to a vial of cefazolin, 1 g (powder for injection).</td>
</tr>
<tr>
<td>b. Dissolve. Take 5 mL of this solution and add it to 5 mL of artificial tears.</td>
</tr>
<tr>
<td>c. Refrigerate and shake well before instillation.</td>
</tr>
<tr>
<td>2. <strong>Tobramycin</strong> 14 mg/mL or gentamicin 14 mg/mL</td>
</tr>
<tr>
<td>a. Withdraw 2 mL of tobramycin or gentamicin injectable vial (40 mg/mL).</td>
</tr>
<tr>
<td>b. Add 2 mL to a tobramycin or gentamicin ophthalmic solution (5 mL) to give a 14 mg/mL solution.</td>
</tr>
<tr>
<td>3. <strong>Vancomycin</strong> 15 mg/mL, vancomycin 25 mg/mL or vancomycin 50 mg/mL</td>
</tr>
<tr>
<td>a. Add 33 mL of 0.9 percent sodium chloride for injection (no preservatives) or artificial tears to a 500 mg vial of vancomycin to produce a solution of 15 mg/mL.</td>
</tr>
<tr>
<td>b. Add 20 mL of 0.9 percent sodium chloride for injection (no preservatives) or artificial tears to produce a solution of 25 mg/mL.</td>
</tr>
<tr>
<td>c. Add 10 mL of 0.9 percent sodium chloride for injection (no preservatives) or artificial tears to produce a solution of 50 mg/mL.</td>
</tr>
<tr>
<td>b. Refrigerate and shake well before instillation.</td>
</tr>
<tr>
<td>4. <strong>Amikacin</strong></td>
</tr>
<tr>
<td>Intravenous formulation can be used (80 mg/2 cc ampules).</td>
</tr>
<tr>
<td>5. <strong>Trimethoprim/Sulfamethoxazole</strong> 16 mg/mL and 80 mg/mL commercial preparation used.</td>
</tr>
</tbody>
</table>

*(Adapted from Basic Clinical and Science Course 2000-2001, Section 8, External Disease and Cornea. American Academy of Ophthalmology)
Bacterial Keratitis

exquisitely potent against *Neisseria* keratitis. They are well-tolerated and more convenient to use.

Monotherapy is advocated in cases of small corneal ulcers not involving the visual axis.

The patients are followed up daily and detailed drawings and measurements are taken to monitor the progress of the therapy.

This includes the resolution of the parameters which include the lid edema, conjunctival congestion, the ulcer size in mm and the depth, the size of the infiltration, corneal epithelial edema and the size of the hypopyon (if any) (Fig. 7.10).

Due to emergence of methicillin resistant Staphylococcus aureus (MRSA), vancomycin should not be used routinely but should be reserved for very severe or recalcitrant infections.

**ADJUNCTIVE THERAPY**

Topical cycloplegics should be administered along with the antimicrobial therapy. This relieves the ciliary spasm, alleviates pain and prevents the formation of synechiae. Homatropine eye drops 1 percent is used in three to four times daily.

Significant inflammation may cause a rise in intraocular pressure and hence in such cases antiglaucoma medications may be added. These include the use of topical beta blockers such as 0.5 percent timolol maleate or systemic carbonic anhydrase inhibitors such as Acetazolamide in more severe cases.

The precise role of non-steroidal anti-inflammatory agents has not been studied in cases of bacterial keratitis but are best avoided as they may cause increased chances of corneal melting.

**Topical Corticosteroids**

Topical corticosteroids are started 48 hours after the organism have been identified on the culture along with the topical antibiotics. This is done after the fungal organisms as causative agents have been ruled out. Alone with the combination therapy it may decrease the amount of inflammation and reduces chances of corneal scaring. Topical corticosteroids should not be used in cases of corneal thinning for impending perforation.

**MODIFICATION OF THERAPY**

The results of microbial culture an the antimicrobial susceptibility testing data may suggest a modification from the initial therapeutic plan. If the patient is responding clinically to the original therapeutic plan, no modification in antibiotic drugs is instituted. However, if the patients is not responding to the therapy or there is worsening of the clinical features, the antibiotic drugs may be changed according to the culture sensitivity reports.

If microbial culture fails to grow an organism, topical medications are stopped for 24 hours before a re-scare is done and cultures are sent again.

**SPECIFIC DRUGS**

Specific drugs may be used when the keratitis occurs due to specific agents. For *Nocardia* keratitis combination
trimethoprim (16 mg/ml) and sulfametoxazole (80 mg/ml) is the treatment of choice. Alternatively, sulfonamides, tetracyclines, erythromycin or amikacin may be used.

In cases of acid fast-stained positive corneal scrapings topical amikacin 10 to 20 mg/ml one drop every hour is indicated. Systemic amikacin may be used in cases of corneal perforation or scleral involvement. More recently, the fluoroquinolones have found to be effective against mycobacteria and can be used as a first line of treatment.

**Subconjunctival Route**

Subconjunctival antibiotics can produce high corneal drug levels. The injections are painful and anxiety provoking. We do not advocate the use of subconjunctival antibiotics as these injections may result in subconjunctival hemorrhage, and if repeated, may lead to subconjunctival fibrosis.

**SYSTEMIC THERAPY**

Systemic antibiotics are started along with the topical antibiotics in cases of severe keratitis with scleral melting, impending perforation, frank perforations which have a propensity for intraocular spread and also when the infection occurs in children due to *H. influenza* and *P. aeruginosa*.

**SIGNS OF HEALING**

The signs of healing of bacterial keratitis or response to therapy include the following: The signs and symptoms decrease and visual acuity continues to improve. The stromal infiltrates consolidate and the anterior chamber reaction decreases. Epithelialization is complete and necrotic stroma is replaced by scar tissue laid down by fibroblasts. Vascularisation occurs and following complete healing the vessels regress completely but sometimes leave “ghost vessels” which are visible through indirect illumination.

**SIGNS OF PROGRESSION/NON-RESPONSE**

Signs of progression include increase in the size of the infiltrate, epithelial defect, height of hypopyon, corneal thinning and eventually perforation.

When culture results are negative but infection is still suspected, and the progression of the disease is such that antibiotic treatment can be suspended temporarily, it is wise to discontinue antibiotics and obtain specimens for re-culture. Antibiotics may then be re-instituted on an empirical basis if there is clinical deterioration until an organism is identified to allow for more specific treatment.

**SURGICAL THERAPY**

The surgical modalities to treat bacterial corneal ulcer include the use of cyanoacrylate glue, patch grafts and therapeutic keratoplasty.

**TISSUE ADHESIVES**

Cyanoacrylate glue to treat small perforations (less than 3 mm), progressive stromal keratolysis and thinned descemetocytes (Figs 7.11A and B). The tissue adhesive is known to have anti-bacterial activity. However, since it is toxic to the corneal endothelium, it should be used with caution. It restores the integrity of the cornea, till the antimicrobial therapy can reduce the ulceration size. The edges and the bed of the site of the perforation is dried completely before application of the glue following which a therapeutic bandage contact lens is placed.

**PATCH GRAFTS**

Patch grafts of upto 5 mm diameter may be used to debulk the cornea and to remove the clinically visible margin of the infected area (Fig. 7.12). It is preferable to encompass 1 mm of the normal cornea in the trephinated area.

**THERAPEUTIC KERATOPLASTY**

If there are large areas of perforation or necrotic tissue, a therapeutic keratoplasty is indicated. Pre-operatively, maximal antibiotic therapy to eradicate infection and to reduce inflammation is recommended. It is preferable to encompass one mm of the normal cornea in the trephinated area (Figs 7.13A and B). Further, the corneal button obtained should be sent for microbiological culture and histopahological examination. Post operatively, systemic antibiotic therapy should also be given along with the topical antimicrobial agents and topical corticosteroids.
Bacterial Keratitis

**Figure 7.11A:** Impending perforation (Courtesy: Medical Photographic Imaging Centre, Royal Victorian Eye and Ear Hospital, Melbourne)

**Figure 7.11B:** Glue applied (Courtesy: Medical Photographic Imaging Centre, Royal Victorian Eye and Ear Hospital, Melbourne)

**Figure 7.12:** Patch graft in a case of perforated corneal ulcer

**Figures 7.13A and B:** Perforated corneal ulcer (A) therapeutic keratoplasty done (B)
Types of Microbial Keratitis

References


Fungal keratitis is one of the most difficult forms of microbial keratitis for the ophthalmologist to diagnose and treat successfully.\textsuperscript{1,2} It is more common in the developing countries as compared to the developed countries. Problems encountered in cases of fungal keratitis include establishing the correct clinical diagnosis and obtaining confirmation of the fungal organisms on laboratory diagnosis. Problems related to therapy include suboptimal penetration of the anti-fungal drugs, difficulty in preparation and availability of the anti-fungal medications.

The treatment of fungal keratitis is quite challenging. Generally, prolonged and intensive topical, intracameral and systemic anti-fungal therapy is required to eradicate the fungal infections, and surgical intervention in the form of penetrating keratoplasty, vasculoplasty or cryotherapy may be undertaken when the medical therapy fails.

**Epidemiology**

**INCIDENCE**

The incidence of fungal keratitis is low (6 to 20\%) as compared to bacterial keratitis in various studies of microbial keratitis especially in the developed countries.\textsuperscript{2} However, the incidence of fungal keratitis may be greater in developing countries where it has been reported in almost half of the cases of microbial keratitis.\textsuperscript{3}

**GEOGRAPHICAL DISTRIBUTION**

Worldwide *Aspergillus* species is the most common fungus responsible for fungal keratitis.\textsuperscript{4} In northern United States, *Candida* species and *Aspergillus* species is the most common cause of fungal keratitis whereas in southern United States *Fusarium* species is the most common organism.\textsuperscript{4,6} *Fusarium solani* was the most frequently isolated organism earlier, whereas in the more recent series, *Fusarium oxysporum* is more common organism (37\%) as compared to *Fusarium solani* (24\%).\textsuperscript{1,7}

Fungal keratitis is a major blinding eye disease in Asia. One of the studies from south India has reported that 44 percent of all corneal ulcers were due to fungi,\textsuperscript{8} whereas its prevalence has been reported to be 17 percent in Nepal,\textsuperscript{9} 36 percent in Bangladesh,\textsuperscript{10} 37.6 percent in Ghana as opposed to 35 percent in Florida.\textsuperscript{11} In India, the most common isolated organism was *Aspergillus* sp. (27 to 64\%) followed by *Fusarium* sp. (6 to 32\%) and *Penicillium* sp. (2 to 29\%).\textsuperscript{3,8} Fungal keratitis is usually seen in the rural areas and warm climates.

**AGE DISTRIBUTION**

Approximately, 65 percent of the patients are in the age group 21 to 50 years, although it has been reported in extremes of age also.\textsuperscript{2}

**SEX DISTRIBUTION**

In general, fungal keratitis does not have any gender predilection, although it has been reported to occur more commonly in males than females in the ratio varying from 1.5:1 to 4.5:1.\textsuperscript{2}

**SEASONAL VARIATION**

A higher preponderance of these cases occurs during monsoons and early winter because of the high humidity found during these months.\textsuperscript{12} A higher incidence has also been reported during harvest seasons, springs, and early winter, probably because of a larger number of vegetative injuries during these seasons.

**Risk Factors**

Various risk factors, which have been incriminated in the causation of fungal keratitis, may be ocular or related to the systemic status of the patient.
Types of Microbial Keratitis

### TABLE 8.1
Risk factors for the development of fungal keratitis

<table>
<thead>
<tr>
<th>OCULAR FACTORS</th>
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<tbody>
<tr>
<td>Trauma</td>
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<tr>
<td>Chronic corneal inflammation</td>
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<tr>
<td>Herpes simplex</td>
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<tr>
<td>Herpes zoster</td>
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<tr>
<td>Vernal allergic conjunctivitis</td>
</tr>
<tr>
<td>Ocular surface problems</td>
</tr>
<tr>
<td>Dry eye</td>
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<tr>
<td>Bullous keratopathy</td>
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<tr>
<td>Exposure Keratopathy</td>
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<tr>
<td>Contact lens wear</td>
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<tr>
<td>Drugs</td>
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<tr>
<td>Corticosteroids</td>
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<tr>
<td>Anesthetics</td>
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<tr>
<td>Corneal surgery</td>
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<tr>
<td>Penetrating Keratoplasty</td>
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<tr>
<td>Refractive surgery</td>
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<table>
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<tr>
<th>SYSTEMIC FACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>HIV positive patients</td>
</tr>
<tr>
<td>Leprosy</td>
</tr>
</tbody>
</table>

**OCULAR FACTORS**

Various ocular factors that may predispose to the occurrence of fungal keratitis include trauma, contact lens wear, medications and prior ocular surgery (Table 8.1).\textsuperscript{13-15} Fungal keratitis also occurs more commonly in cases of previously compromised corneas or corneal scars.

**Trauma**

The initiating trauma in cases of fungal keratitis is usually trivial and is associated with vegetable or organic matter\textsuperscript{13} (Fig. 8.1). It most often occurs outdoors and involves plant matter such as leaves and paddy grains. It may also occur after injury with mud or sand and even injury from animal origin such as due to cow dung and cow tail and may occur even with metal pieces.

Fungal keratitis should be especially suspected in children especially in cases when trauma with organic matter occurs. In one series fungal keratitis composed 18 percent of all cases of keratitis cultured in children.\textsuperscript{16} All children with a suspected keratitis should be cultured for fungi.

**Chronic Corneal Inflammation**

Less common risk factors for fungal keratitis include chronic keratitis due to vernal or allergic keratoconjunctivitis and neurotrophic ulcers secondary to varicella zoster or herpes simplex viruses ocular surface disorders, dry eye, Steven Johnson syndrome and bullous keratopathy. It may also occur in cases of ocular atopy.\textsuperscript{17}

**Contact Lens Wear**

Fungal keratitis following contact lens wear is rare and is responsible for only 3-29 percent of cases associated with contact lens wear.\textsuperscript{13,18,19} Filamentous fungi are more commonly associated with cosmetic or aphakic lens wear, and yeasts are more frequently associated with therapeutic lens use.\textsuperscript{18-20}

**Drugs**

A history of use of prior medications should be obtained in all cases. Most patients of fungal keratitis are already on treatment with topical antibiotics or corticosteroids. The indiscriminate use of topical antibiotics and topical corticosteroids has been associated with fungal keratitis especially in developing countries where the drugs are available over the counter without any prescription. The use of latter has been particularly associated with the worsening of fungal keratitis.\textsuperscript{21} They appear to activate and increase the virulence of fungi.\textsuperscript{21} The infiltrates are more extensive in such cases and response to anti-fungal therapy is more sluggish. Systemic use of corticosteroids may predispose the patient to fungal keratitis as the immune response of the host is suppressed. The use of traditional eye medicines may also predispose to the occurrence of fungal keratitis (Fig. 8.2).
Fungal keratitis may occur virtually after any ocular surgery, which includes keratoplasty (Fig. 8.3), cataract surgery, and refractive surgery. Predisposing factors for the development of fungal keratitis in patients after penetrating keratoplasty include suture related problems, topical steroid and antibiotic use, failed grafts and persistent epithelial defects. The contamination of donor corneas with fungal organisms may occur as no anti-fungal therapy is routinely used to decontaminate the donor eyes.

Cases of delayed fungal keratitis have also been reported months or years after the refractive surgery was performed. This was also true for radial keratotomy and now more recently photorefractive keratotomy and laser in situ keratomileusis (Fig. 8.4). It may appear in the early post-operative period or later. The early appearance is usually associated with the surgical contamination whereas the late appearance is often related to trauma.

**SYSTEMIC FACTORS**

Some systemic diseases associated with immunosuppression may increase the risk for the development of fungal keratitis. These include diseases such as diabetes mellitus, patients with chronically debilitated diseases who are hospitalized in the intensive care units, HIV positive patients and cases of leprosy (Table 8.1).
Types of Microbial Keratitis

Fungal isolates can be classified into four groups: Moniliaceae, which are nonpigmented filamentary fungi including *Fusarium* sp and *Aspergillus* sp; Dematiaceae, which are pigmented filamentary fungi including *Curvularia* sp and *Lasiodiplodia* sp; yeasts, which include *Candida* sp; and other fungi.

Fungi are a part of normal microbial environment and in the absence of a precipitating event, rarely cause infection in human cornea. They gain access into the corneal stroma through a defect in the epithelium. This defect may be due to external trauma, including contact lenses, a compromised ocular surface, or previous surgery. In the stroma, they proliferate and can cause tissue necrosis and a host inflammatory reaction. These organisms can penetrate deep into the stroma even through an intact Descemet’s membrane. When the organisms gain access into the anterior chamber or iris, their eradication becomes difficult. Blood-borne growth inhibiting factors may not reach the avascular tissue such as the cornea, anterior chamber and sclera, and hence the fungi continue to multiply and persist despite treatment. This is also the basis for the vasculoplasty procedure, which in the form of a conjunctival flap helps in healing of the fungal keratitis by bringing the blood-borne growth-inhibiting factors from the vascular areas towards the avascular areas.

### Clinical Features

In general, the symptoms of fungal keratitis are less severe as compared to bacterial and viral keratitis. Fungal keratitis may be associated with certain general symptoms and signs and also specific features which may clinically point to an etiological agent (Table 8.3).

### GENERAL SYMPTOMS

The onset of fungal keratitis is almost always insidious. Symptoms are usually non-specific, although possibly more prolonged duration (5 to 10 days). Patients generally complaint of a foreign body sensation for several days with a slow onset of increasing pain and diminution of vision especially if the keratitis involves the visual axis.

### GENERAL SIGNS

On slit lamp examination, the infiltrates appear grayish white or yellowish white and the base of the ulcer is

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**TABLE 8.2**

<table>
<thead>
<tr>
<th>Fungi causing human keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. FILAMENTOUS</strong></td>
</tr>
<tr>
<td><strong>A. SEPTATED</strong></td>
</tr>
<tr>
<td>1. Nonpigmented</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
</tr>
<tr>
<td>solani, oxysporum, moniliforme, episphaesia, nivale</td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
</tr>
<tr>
<td>fumigatus, flavus</td>
</tr>
<tr>
<td><em>Acremonium (Cephalosporium)</em></td>
</tr>
<tr>
<td><em>Paecilomyces</em></td>
</tr>
<tr>
<td><em>Penicillium</em></td>
</tr>
<tr>
<td>2. Pigmented (Dematiaceous)</td>
</tr>
<tr>
<td><em>Curvularia</em></td>
</tr>
<tr>
<td>senegelensis, verruculosa, pallescens</td>
</tr>
<tr>
<td><em>Lasiodiplodia</em></td>
</tr>
<tr>
<td>theobromae</td>
</tr>
<tr>
<td><em>Alternaria</em></td>
</tr>
<tr>
<td><em>Cladosporium</em></td>
</tr>
<tr>
<td><em>Cellerotrichum</em></td>
</tr>
<tr>
<td><em>Drechslera (Helminthosporium)</em></td>
</tr>
<tr>
<td><strong>B. NONSEPTATED</strong></td>
</tr>
<tr>
<td><em>Rhizopus (mucormycosis)</em></td>
</tr>
<tr>
<td><strong>II. YEAST</strong></td>
</tr>
<tr>
<td><em>Candida</em></td>
</tr>
<tr>
<td>albicans, parapsilosis, krusei, tropicalis</td>
</tr>
</tbody>
</table>

**TABLE 8.3**

<table>
<thead>
<tr>
<th>Clinical features of fungal keratitis</th>
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</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
</tr>
<tr>
<td>Foreign body sensation</td>
</tr>
<tr>
<td>Gradually increasing pain</td>
</tr>
<tr>
<td>Diminution of vision</td>
</tr>
<tr>
<td><strong>Signs</strong></td>
</tr>
<tr>
<td>Nonspecific</td>
</tr>
<tr>
<td>Conjunctival injection</td>
</tr>
<tr>
<td>Epithelial defect</td>
</tr>
<tr>
<td>Specific</td>
</tr>
<tr>
<td>Feathery margins</td>
</tr>
<tr>
<td>Elevated edges</td>
</tr>
<tr>
<td>Rough texture</td>
</tr>
<tr>
<td>Satellite lesions</td>
</tr>
<tr>
<td>Gray/Brown pigmentation</td>
</tr>
<tr>
<td>Collar button configuration</td>
</tr>
<tr>
<td>Fixed hypopyon</td>
</tr>
</tbody>
</table>
often filled with soft, creamy and raised exudates (Fig. 8.5). The fungal ulcers have characteristic findings, which include elevated areas, hyphate (branching) ulcers, irregular feathery margins, a dry rough texture (Fig. 8.6), and satellite lesions. Feathery borders or hyphate edges are seen in 70 percent of the patients and satellite lesions are seen in 10 percent of the patients. Hypopyon is generally fixed and may be present in 45 to 66 percent of the cases. Hypopyon is generally fixed and may be present in 45 to 66 percent of the cases. An immune ring, endothelial plaque and a posterior corneal abscess may be present rarely (Fig. 8.7).

**SPECIFIC FEATURES**

Each case of fungal keratitis may exhibit these basic features but may differ in the clinical course depending upon the etiological agent. The most common manifestations of culture-proven mycotic keratitis are reported to be a gray or dirty-white surface, anterior-chamber cellular reaction, irregular feathery margins, elevated borders, dry rough texture, satellite lesions, Descemet’s folds, hypopyon, ring infiltrate, endothelial plaque, and keratitic precipitates.

**Dematiaceous Fungi**

The appearance of macroscopic brown pigmentation in fungal keratitis may be due to the presence of a dematiaceous fungus (*Curvularia lunata*) (Fig. 8.8). The pigmentation has been related to the alteration in the melanin metabolism and when present indicates a more superficial infection, low virulence of the organism and
Types of Microbial Keratitis

less inflammatory reaction. In some cases of dematiaceous keratitis where absence of pigmentation is correlated with a masking by a more intense inflammatory reaction. The presence of an intact epithelium with a deep stromal infiltrate may also be found in fungal keratitis.

Fusarium

Fusarium solani keratitis has a more severe course so that deep extension and perforation may occur in few weeks (Fig. 8.9). Aspergillus species on the other hand, causes a less severe and not so rapidly progressive keratitis, which is amenable to therapy (Fig. 8.6).

Yeast

A “collar button” configuration is typical of the keratitis caused by yeasts, which is often associated with a small ulceration and an expanding discreet stromal infiltrate (Fig. 8.10). The stromal keratitis caused by C. albicans and related fungi resembles bacterial keratitis, with an overlying epithelial defect, a more discrete infiltrate, and slow progression. Such ulcers frequently occur in eyes with preexisting corneal disease and in areas of exposure typically at the junction of the superior two-thirds and inferior one-third of the cornea.

Laboratory Diagnosis

Corneal ulcer scrapings from the ulcer edge and the base form the mainstay of the diagnosis of a case of fungal keratitis. Corneal scraping with a spatula or a surgical blade is preferred to the use a calcium alginate, dacron/rayon swab, or a sponge-type material. The organisms may be deeper in the tissues and may not be accessible to a more superficial scraping. Corneal scraping not only provides diagnostic clues but also may be therapeutic as it also aids in the initial debridement and debulking of the organisms. Further, it also breaches the epithelium, which may provide a barrier to the penetration of the anti-fungal agents.

Cultures should also be sent from topically applied medications, cosmetics, contact lenses and their storage and cleaning solutions, wherever indicated. In case of deeper lesions in fungal keratitis, a surface is passed through the lesion and may be sent for culture examination.

Apart from this anterior chamber tap and corneal biopsy may be done especially in cases of deep keratitis and endothelial plaques.

Laboratory diagnosis of fungal keratitis primarily includes direct microscopy, fungal cultures and newer diagnostic modalities such as polymerase chain reaction (PCR) and confocal microscopy.

DIRECT MICROSCOPY

Direct microscopy uses KOH wet mount preparation and smears, which are stained by Gram and Giemsa stain.

KOH Wet Mount Preparation

In our experience, 10 percent KOH wet mount is simple, cheap, rapid and easy to interpret and is particularly useful in tropical countries. KOH smear has a sensitivity of 72.2 to 91 percent.
Gram’s Stain
Gram stain is equally sensitive in detecting fungal organisms. Gram stain identifies fungal species in 31.6 to 98 percent.\textsuperscript{34,35}

Giemsa Stain
Giemsa stain identifies fungal elements in 27 to 85 percent of the cases.\textsuperscript{34,35}

Lactophenol Cotton Blue
Lactophenol cotton blue has a sensitivity of 70 to 80 percent in cases of fungal keratitis.\textsuperscript{36}

Grocott’s Methenamine-silver Stain
Grocott’s methenamine-silver staining has a sensitivity of 89 percent. A shortened method of Grocott’s methenamine-silver staining is also available which is reliable. The stain can also be reused for re-staining the Gram and the Giemsa stained slides and often reveals hyphae not visualized by other stains.

Calcofluor White
Calcofluor white has a sensitivity of 80 to 90 percent. Excellent results can be achieved when the nonspecific fluorescent stain calcofluor white was used to stain corneal scrapes or biopsy specimens prior to direct microscopic examination.\textsuperscript{36}

<table>
<thead>
<tr>
<th>Staining Technique</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td>Gram’s stain</td>
<td>31.6% - 98%</td>
</tr>
<tr>
<td>KOH wet mount</td>
<td>72.2% - 91%</td>
</tr>
<tr>
<td>Gomori Methenamine stain\textsuperscript{37}</td>
<td>56%</td>
</tr>
<tr>
<td>Giemsa smear</td>
<td>27% - 85%</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>76%</td>
</tr>
</tbody>
</table>

Fungal Keratitis

A definitive diagnosis of fungal keratitis is made if
1. Corneal scrapings reveal fungal elements in smears.
2. Fungus grows in more than one medium in the absence of fungus in smears.
3. Fungus grows on a single medium in the presence of fungus in smears.
4. Confluent growth of fungus appears at the inoculated site on a single solid medium.

A fungus grown on the primary isolation medium may be sub-cultured onto a potato dextrose agar (PDA) medium and incubated for a period of 10 days to facilitate sporulation. Following adequate growth of the fungal isolate on PDA, the identification may be carried out based on its macroscopic and microscopic features (Fig. 8.11).

Positive cultures should be expected in 52 to 68 percent of cases. Initial growth occurs within 72 hours in 83 percent of cultures and within 1 week in 97 percent of cultures.\textsuperscript{11,29,30} Most in fact are visible with dissecting microscope or naked eye within 36 hours. But we should wait for at least a week before declaring a culture negative for fungi. Because both yeast and hyphae readily grow in sheep blood agar and Sabouraud dextrose agar at room temperature, other media such as brain heart infusion may not be used routinely. Increasing the humidity of the medium by placing the inoculated agar plates in plastic bags has also been recommended for enhancement of fungal growth.

NEWER DIAGNOSTIC MODALITIES

More recent methods for the identification of fungi, although still not widely available, include immuno-
Types of Microbial Keratitis

fluorescence staining, electron microscopy, polymerase chain reaction, and confocal microscopy. These newer diagnostic modalities may not be available at all places.

Polymerase Chain Reaction

Gaudio et al have reported that PCR and culture results matched in 74 percent of the cases. This technique requires only 4 hours to obtain the results which is quicker than the 2 days to 2 weeks required by culture methods and in future may become a valuable adjunctive tool for the diagnosis of fungal keratitis, although it can not replace culture methods as the possibility of false-positive results needs to be considered.

Confocal Microscopy

Confocal microscopy has recently been used in cases of fungal keratitis, which helps to identify the hyphal elements and the yeasts. Confocal microscopy is an imaging technique that allows optical sectioning of almost any material, with increased axial and lateral spatial resolution and better image contrast, which may be useful for the identification of corneal pathogens in the early stages of infection. In clinical keratitis due to Aspergillus sp., fungal hyphae can be imaged as high-contrast filaments, 60 to 400 Å long and 6 Å wide. In patients with mycotic keratitis, in vivo scanning slit confocal microscopy helps in establishing the diagnosis and demonstrating non-responsiveness to medical therapy by showing an increased load of fungal filaments, therefore aiding the treatment decision. Thus, confocal microscopy is a potentially useful, noninvasive technique to determine the presence of fungal hyphae in vivo within the human cornea. Limitations in the use of this technique for routine diagnosis relate to instrument configuration, movement of either the tissue or the microscope, difficulty in reproducibly returning to the area of interest for serial examination, lack of a distinctive morphology of some pathogens, and limited resolution of the microscope.

KERATECTOMY/BIOPSY

If corneal scrapings for smears and cultures are negative, a diagnostic superficial keratectomy or corneal biopsy may become necessary. The biopsy can be performed in the minor operating room or at the slit lamp under topical anesthesia using 0.5 percent proparacaine and 2 percent xylocaine eye drops. Other anesthetic agents, which may be used, are tetracaine, or cocaine. In some cases, eyelid and retrobulbar anesthesia may be required. Under the microscope, a round 2 to 3 mm sterile disposable dermatologic trephine is used and partial thickness trephination is done in such a manner so that it encompasses both the clinically infected area and the adjacent clear cornea. Care is taken to avoid the visual axis, if possible. The base is then undermined with a surgical blade to complete the lamellar keratectomy. The corneal biopsy specimen should be sent for smears, cultures, and histopathological examination.

Corneal biopsy is considered to be superior to corneal scraping for the isolation of the fungal organisms.

Management

The management consists of medical therapy and surgical intervention in cases, which are non-responsive to medical therapy.

MEDICAL THERAPY

Prompt and appropriate anti-fungal therapy is the mainstay of the treatment of fungal keratitis. Anti-fungal therapy should only be instituted where corneal scraping reveals the presence of fungal elements or cultures reveal the presence of fungal organisms at 36-48 hours. We do not recommend an empirical anti-fungal therapy based on the clinical evidence of fungal keratitis alone.

Since the corneal epithelium serves as a barrier to the penetration of most tropical anti-fungal agents, debridement of the corneal epithelium is an essential component of the medical management of fungal keratitis especially early in the course of treatment.

The topical anti-fungal therapy is the mainstay of fungal keratitis.

Topical Antifungal Agents

Commercially available natamycin 5 percent suspension is the initial drug of choice for fungal keratitis.

It should be given hourly during the day and two hourly during night time. In addition to the anti-fungal drugs a broad-spectrum antibiotic such as a fluoroquinolone may be given to prevent secondary bacterial infection. Additionally, cycloplegics such as homatropine eye drops may be given three times a day to relieve the component of iridocyclitis along with the
anti-glaucoma medications in cases where the intraocular pressure is high on digital tonometry. The eye should be examined twice daily preferably under the slit lamp. Once the infiltrate started resolving, the frequency of topical natamycin is reduced to 2-hourly until the completion of resolution (Figs 8.12A and B). The natamycin should be continued for 2 weeks after the resolution of infection in all cases.

If worsening of the keratitis is observed on topical natamycin, topical amphotericin B 0.15 percent with or without fluconazole 2 percent may be added as a second agent. In cases of proven *Candida* sp., amphotericin B 0.15 percent or fluconazole 0.3 percent is the first drugs of choice.

Amphotericin B has to be prepared extemporaneously. It is available as a systemic preparation. In order to prepare the topical form, the compound has to be diluted with dextrose or distilled water to obtain a concentration of 0.15 percent. It is not effective against *Fusarium* species.

The efficacy of Econazole 1 percent against filamentous fungi has been found to be equivalent to natamycin 5 percent. Clotrimazole is available in 1 percent topical drops and ointment form and has been used in the treatment of fungal keratitis. The imidazoles (ketocnazole and miconazole) are used systemically for the treatment of keratomycosis because of their relatively reduced systemic toxicity. Fluconazole is a fungistatic bitriazole which is used topically and systemically in the treatment of *Candida* and *Aspergillus* keratitis. It does not show encouraging results against *Aspergillus* species and *Fusarium* species.

A new azole antifungal agent, Voriconazole, is derived from Fluconazole and exhibits a wider spectrum of activity against *Candida*, *Aspergillus* and *Fusarium*. It exerts its effect from inhibition of cytochrome P450-dependant 14 alpha sterol demethylase, an enzyme involved in the ergosterol biosynthetic pathway. The minimal inhibitory concentration of voriconazole (0.5 μg/ml) is less as compared to other imidazoles.

Topical voriconazole 1% has to be prepared in pharmacy as it is not commercially available and is given in recalcitrant fungal keratitis if there is not response to topical natamycin and amphotericine B therapy.

Echinocandins have also been used for systemic mycoses. These agents target the synthetic cell wall enzyme complex beta-1, 3-D glucon synthase. The antifungal spectrum is however limited to *Candida* and *Aspergillus* species.

**Response to Therapy**

Since fungal keratitis responds slowly over a period of weeks, clinical signs of improvement should be noted which include the following: diminution of pain, decrease in size in size of infiltrate, disappearance of satellite lesions, rounding out of the feathery margins of the ulcer and hyperplastic masses, or fibrous sheets.

**Duration of Treatment**

In general the duration of treatment is longer than that for cases of bacterial keratitis. The clinician must determine the length of treatment for each individual case.
based on clinical response. The duration of the treatment for topical treatment has not been firmly established clinically or experimentally and varies from 30 to 39 days. Problems that can rise from prolonged treatment are due to toxicity. The inflammatory response from this toxicity can be confused with persistent infection. If toxicity is suspected and if adequate treatment has been given for at least 4 to 6 weeks, treatment should be discontinued and the patient carefully observed for evidence of recurrence.

**Drug Interactions**

Several topical anti-fungal medications act synergistically against a particular fungal organism. In clinical series more than one concurrent topical anti-fungal has been needed 5 percent of the time. Synergistic drugs include a combination of amphotericin B and fluocytosine, (for *Candida* keratitis) and a combination of natamycin and ketoconazole (for *Aspergillus* keratitis). Likewise, experimental models have demonstrated the potential antagonism between anti-fungals such as amphotericin B and the imidazoles.

**Drug Resistance**

Resistance to anti-fungal agents is rare and generally occurs when they are used for systemic mycoses. Competition for volume in the pre corneal tear film and washout may be of more concern when using two topical anti-fungals.

**Systemic Antifungal Agents**

Treatment with a systemic anti-fungal agent is recommended in cases of very large ulcers, severe deep keratitis, scleritis and endophthalmitis. Systemic antifungals also may be used as prophylactic treatment after penetrating keratoplasty for fungal keratitis.

The drugs, which have been used systemically, include ketoconazole (oral), miconazole (intravenous), itraconazole (orally 200 mg/day) and fluconazole (orally 200 mg/day). More recently oval voriconazole 200 mg bd has shown good results in recalcitrant fungal keratitis.

The most frequently used oral anti-fungal is ketoconazole, which is given in the dose of 600 mg per day. It is mandatory to assess liver function tests every 2 weeks after starting ketoconazole. Systemic therapy is given for a period of 6 to 8 weeks.

**Topical Corticosteroids**

Topical corticosteroid in the treatment of fungal keratitis should not be used. The topical corticosteroids worsen the disease when given alone and adversely influence the efficacy of natamycin, fluocytosine and miconazole when given in combination.

**Intracameral Therapy**

Intracameral amphotericin B may be a useful modality in the treatment of severe keratomycosis not responding to topical natamycin. It ensures adequate drug delivery into the anterior chamber and may be especially useful to avoid surgical intervention in the acute stage of the disease (Figs 8.13A and B).

The procedure should be performed under strict aseptic conditions. If the infection involves the anterior capsule of the lens, care should be taken to avoid injury to the lens. Patients with deep keratomycosis unresponsive to conventional medical treatment are candidates for intracameral injections of 5 μg to 7.5 μg amphotericin B in 0.1 mL 5 percent dextrose. Injections can be repeated in case of inadequate response.

**Intrastromal Therapy**

A recent modality advocated for non healing fungal corneal ulcers is the use of intracorneal Amphotericin B injection in 5-7.5 μg dosage, given in the vicinity of the stromal site of fungal growth. This would raise the local concentration of the antifungal agent enough to be effective in the eradication of the deep corneal infection. This approach proves effective, with total elimination of the infection (Figs 8.14A and B). The intrastromal injection can be repeated after a period of 48 to 72 hours. Although further experience is required, intrastromal corneal injections of Amphotericin B may offer a good choice for recalcitrant cases of fungal keratitis.

**SURGICAL THERAPY**

**Debridement**

Daily debridement with a spatula or blade is the simplest form of surgical intervention and is usually performed at the slit lamp under topical anesthesia. Debridement is performed every 24 to 48 hours and works by debulking organisms and necrotic material and by enhancing the penetration of the topical antifungal.
Biopsy

A biopsy may be used not only for the diagnosis but also as a therapeutic intervention.

Therapeutic Keratoplasty

Approximately one third of fungal infections result in either medical treatment failures or corneal perforations. The main goals are to control the infection and maintain the integrity of the globe.

Most retrospective series indicate that keratoplasty was performed within 4 weeks of presentation, primarily because of medical treatment failures; in some cases it may be required because of recurrence of infection.

When progression of the keratitis is noted, penetrating keratoplasty should be performed. If the infectious process is allowed to progress until it involves the limbus or sclera, unfavorable outcomes secondary to scleritis, endophthalmitis, and recurrence are more common. Therapeutic keratoplasty should be performed in cases of impending perforations, frank perforations > 2 mm or if there is no response to therapy.

The technique of the keratoplasty is similar to that performed for other forms of microbial keratitis. The size of the trephination should leave a 1 to 1.5 mm clear zone of clinically uninvolved cornea to reduce the possibility of residual fungal organisms peripheral to the trephination. Interrupted sutures with slightly longer
Types of Microbial Keratitis

bites should be used to avoid cheese wiring of the suture if the edge of the recipient becomes involved with a persistent organism. Irrigation of the anterior segment should be performed to eliminate any organisms. As far as possible the lens should be left untouched to prevent the spread of infection in the posterior segment. However, if affected the intraocular structures including the iris, lens, and vitreous may be excised. The specimens removed should be submitted to both the microbiology and pathology laboratories for culture and fixed section examination. If involvement of intraocular structures or endophthalmitis is suspected, an antifungal agent should be injected which includes amphotericin B (5 μg/0.1 ml) or miconazole (25 μg/0.1 ml).

It is mandatory to submit surgical specimens from cases of microbial keratitis for histopathologic examination especially if the microbiologic diagnosis is not known. Histopathologic examination of corneal buttons can reveal the presence of fungal elements in 75 percent patients. It has been shown that 59 percent of corneas infected by fungi are still culture-positive at the time of keratoplasty, with 90 percent of eyes exhibiting hyphal elements on pathologic examination. Fungal hyphae usually lie parallel to the corneal surface and lamellae. A vertical or perpendicular arrangement of fungal hyphae in the corneal stroma has been associated with increased virulence and in patients on topical corticosteroid therapy.

After penetrating keratoplasty, topical antifungal agents should be continued to prevent recurrence of infection. Postoperatively, systemic ketoconazole or fluconazole may be used in addition to topical antifungal agents. If the pathology laboratory reports that no organisms were seen at the edge of the corneal specimen, antifungals could be stopped after 2 weeks and the patient followed carefully for recurrences. A report from the microbiology laboratory regarding growth of organisms from the corneal or intraocular tissues should indicate the need for more prolonged topical and systemic anti-fungal therapy, possibly for 6 to 8 weeks.

At the time of keratoplasty, if the infection has been controlled clinically, topical corticosteroids may be used. If it is not known whether the infection is controlled, corticosteroids should be avoided during the early postoperative period. Although the main goal of penetrating keratoplasty in fungal keratitis is to eliminate the infecting organism, a secondary goal is the maintenance of a clear corneal transplant for optical reasons (Figs 8.15A and B). Even if graft failure or rejection occurs, the patient can undergo a second optical keratoplasty once the rejection is controlled. The effects of other immunosuppressive medications such as cyclosporin A and its effect on fungal growth has not been well documented clinically.

References

Types of Microbial Keratitis

Viral Keratitis is the commonest cause of keratitis in the developed world. The virus can infect individual layers of the cornea or in more severe form it may involve all the layers of cornea. The various viral infections that can affect the cornea can broadly be grouped under the following categories herpes simplex keratitis, varicella zoster induced keratitis and the adenoviral keratitis.

**Herpes Simplex Virus**

The herpes simplex virus is a DNA virus, which belongs to the Herpesviridae family of viruses. The virus specific antigens differentiate HSV into two types that is the herpes simplex virus type-1 (HSV-1) and the herpes simplex virus type-2.

**Epidemiology**

Herpes simplex keratitis is the most common infective cause of blindness in many developed countries. The ocular disease affecting the cornea may be classified into primary or recurrent.

The incidence of all episodes of herpes simplex keratitis was repaired as 20.7 cases/100,000 population and the prevalence of ocular HSV disease in the community was calculated at 149 per 100,000 population in one study. The incidence of new episodes of HSV was reported to be 8.4/100,000/year.\(^1\)

The disease may occur bilaterally in 11.9 percent patients and is more common in atopes and immunosuppressed.\(^2\)

The recurrences are generally caused by the same strain of virus as the initial infection. Recurrences generally occur in 20 percent patients by 2 years, 40 percent by 5 years and 67 percent by 7 years.\(^1\) Generally the recurrence of the same type of ocular disease occurs that is the patients with epithelial keratitis have an epithelial recurrence and that of stromal keratitis have stromal recurrence.\(^2\)

**Pathophysiology**

**INFECTION**

Herpes simplex virus is a large and complex enveloped virus measuring 150-200 nm. It has a double stranded DNA core, which is surrounded by a protein capsid that is made up of 162 subunits called capsomer. The capsid is surrounded by the tegument, membrane of the infected cell that has been altered by virus-induced proteins.

Humans are the only natural reservoirs of herpes. The sources of infection are by direct contact with infected lesions, by salivary droplets or fomites from children and adults with active disease and also of asymptomatic virus shedding carriers. A patient can also acquire the infection iatrogenically by physician’s unwashed hands or by contaminated Schiotz or applanation tonometer head.

It is estimated that > 90 percent of population have had a type 1 HSV infection during their lifetime, usually during childhood or early adolescence.

The most common type of herpes simplex virus is HSV-1 which causes cold sore or fever blister in the mouth, face and upper body and may affect the eye. HSV-2 causes genital herpes, a sexually transmitted disease. Ocular herpes is caused primarily by HSV-1 and occasionally by HSV type-2 virus.

Primary HSV-1 infection occurs usually in the mucocutaneous distribution of the trigeminal nerve. After the
Types of Microbial Keratitis

primary infection, the virus spreads from the infected epithelial cells to nearby sensory nerve endings and is transported along the nerve axon to the cell body located in the trigeminal ganglion. The virus genome enters the nucleus of a neuron, where it persists indefinitely in a latent state. The primary infection of any of the 3 branches (i.e. ophthalmic, maxillary, mandibular) of cranial nerve V can lead to latent infection of nerve cells in the trigeminal ganglion. Inter-neuronal spread of HSV within the ganglion causes ocular disease even in cases which have not had primary ocular HSV infection.

Infection is spread by direct contact of infectious secretions with epidermis or mucous membrane. HSV-2 may rarely infect the eye by means of direct contact with infectious genital secretions and occasionally is transmitted to neonates as they pass through the birth canal of a mother with genital HSV-2 infection. Recurrent HSV infection traditionally has been thought of as reactivation of virus in the sensory ganglion, which migrates down the nerve axon to produce a lytic infection in ocular tissue. The virus may be present in a latent phase within the corneal tissue, acting as a potential source of recurrent disease and also responsible for the donor-derived HSV in transplanted corneas.

The triggering agents for reactivation of an acute attack of herpes includes fever, hormonal changes, ultraviolet exposure, psychological stress, ocular trauma, immunocompromised and trigeminal nerve manipulation. The excimer laser ablation may also trigger a reactivation of herpetic keratitis.

IMMUNE MECHANISMS

The cell mediated and the humoral immunity are activated which limit the spread of infection and are also responsible for the sequel to the pathologic process. Stromal inflammation and endothelitis occur as a consequence of replicating virus or altered antigenicity of the stromal cells causing immune mediated destruction. Secretion of glycoproteins is responsible for stromal inflammation which occurs in a greater quantity in stromal inflammation as compared to cases in which epithelial involvement occurs alone.

Clinical Features

CONGENITAL OCULAR HERPES

HSV-1 and HSV-2 can be acquired in utero, by transplacental or ascending infection, by exposure to genital lesions during delivery, or postnatally from relatives or attendants. The clinical manifestations of this rare, but devastating disease depend on the stage or trimester when HSV is contracted. Of all the neonatal HSV, 4 percent is acquired during intrauterine life, 86 percent infection occurs at the time of birth and remaining 10 percent occurs in the postnatal period. Congenital HSV infection is characterized by the triad of skin vesicles, eye disease and microencephaly.

NEONATAL HSV KERATITIS

Neonatal HSV infection usually presents as a bilateral disease at 2 days to 2 weeks of age. HSV keratitis in a neonate is invariably associated with conjunctivitis. Keratitis may manifest as diffuse dendritic ulcers, serpiginous epithelial defects or a punctate keratitis. The diagnosis of ocular HSV must be considered in any infant with nonpurulent conjunctivitis or keratitis. Treatment of neonatal ocular herpetic disease comprises of topical antivirals (1% Trifluridine ophthalmic solution or 3% acyclovir ophthalmic ointment) in addition to systemic Acyclovir (2 g/day IV every 8 hourly for 14 days).

PRIMARY OCULAR HERPES

Primary ocular HSV is an acute HSV infection of the non-immune host. It occurs after 6 months of age following decline in maternal antibodies. Most primary infections occur between 1-5 years of age and are subclinical. Only 6 percent of those infected actually develop clinical manifestations, which typically affect the perioral area rather than the eye. Clinically overt disease begins 3-9 days after exposure and usually causes more symptoms than the recurrent disease. It manifests as intense occasionally hemorrhagic, vesicular, periocular dermatitis or blepharitis, follicular conjunctivitis that may be pseudomembranous or geographic ulceration or both, corneal ulceration, iritis and non-suppurative periocular lymphadenopathy.

The primary infection is self-limited with complete recovery and disappearance of virus from initial site of infection in immunocompetent individuals. The site of primary infection determines the pathway of the viral spread and the site of viral latency. The most common site of primary HSV infection in the facial area is that served by maxillary division of trigeminal nerve. This results in latency in the trigeminal ganglion. Herpes simplex virus can also establish latent infection in other neurons of the sensory and autonomic ganglia that supply the affected area. It occurs within 3 weeks of
primary infection whether clinically overt or asymptomatic. This latency is life long and the virus can be reactivated by number of trigger mechanisms. The trigeminal ganglion is the most common site for HSV-1 latency. The superior cervical, vagus, sacral and autonomic ganglia are other sites of latent virus. Apart from the sensory and autonomic ganglia, there are evidences which suggest that cornea can also serve as a non-neuronal site of latency and as a source of future infectious virus during reactivation.5

RECURRENT OCULAR HERPES

The recurrence rate of ocular herpes has been reviewed by Liesegang and is reported to be 36 percent at 5 years and 63 percent at 20 years after primary episode.1 After a second episode, 70-80 percent of patients have recurrence with in 10 years.1 Recurrent herpes simplex can present as blepharitis, conjunctivitis, epithelial keratitis, stromal keratitis, uveitis, trabeculitis and even chorioretinitis.6

Although the mechanism of viral reactivation is not completely understood a variety of trigger factors are recognized which include fever, ultraviolet light, cold wind systemic illness, surgery, menstruation, minor local trauma, immunosuppression from either endogenous disease or iatrogenic drug management of disease and laser photokeratectomy.7

MANIFESTATIONS OF KERATITIS

HSV keratitis is predominantly a unilateral disease and bilateral herpetic keratitis occurs only in 3 percent of patients with ocular HSV infection.7 Bilateral disease is more common in cases of atopy and in younger patients.

HSV keratitis can manifest as one of the following (Table 9.1):

- Infectious epithelial keratitis
- Neurotrophic epithelial keratitis
- Herpetic stromal keratitis
- Endothelitis.

INFECTIONOUS EPITHELIAL KERATITIS

Infectious epithelial keratitis is caused by the presence of live virus. The patient presents with lacrimation, photophobia, irritation and occasionally blurred vision. The corneal lesions may manifest in various forms.

Punctate Keratitis

These lesions begin as punctate or stellate whitish opaque plaques of swollen epithelial cells.

<table>
<thead>
<tr>
<th>Table 9.1</th>
<th>Pathogenesis of herpetic keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical presentation</strong></td>
<td><strong>Pathogenesis</strong></td>
</tr>
<tr>
<td>Infectious epithelial keratitis</td>
<td>Live virus in epithelium</td>
</tr>
<tr>
<td>Neurotrophic keratopathy</td>
<td>Immune response in stroma</td>
</tr>
<tr>
<td>Immune stromal keratitis</td>
<td>Antigen antibody complex mediated</td>
</tr>
<tr>
<td>Necrotizing stromal keratitis</td>
<td>Direct viral invasion</td>
</tr>
<tr>
<td>Endothelitis</td>
<td>Immune reaction involving endothelium</td>
</tr>
</tbody>
</table>

Dendritic Keratitis

The plaques of swollen infected epithelial cells enlarge to form branching dendrites. The epithelium in the center sloughs to form dendritic ulcers. Dendritic ulcers may be single or multiple (Fig. 9.1) and have linear branches with terminal bulbs (Fig. 9.2). The edges of the ulcers are raised and contain replicating virus. These ulcers are usually central or paracentral. The area of dendritic ulceration is typically anesthetic whereas the surrounding cornea may retain normal sensation. These ulcers stain with fluorescein along the length of the lesion whereas the rose bengal stains the devitalized cells and is typically taken up by the swollen epithelial cells at the ulcers border.

![Figure 9.1: Dendritic keratitis due to herpes simplex virus (Courtesy: Medical Photographic Imaging Centre, Royal Victorian Eye and Ear Hospital, Melbourne)
Types of Microbial Keratitis

Geographic Ulcers

Some of the dendrites enlarge and develop into geographic ulcers. These ulcers have amoeboid shape and like dendrites have virus laden devitalized epithelial cells at their margins that take up rose bengal stain (Fig. 9.3). However if viral cultures or polymerase chain reaction (PCR) are to be performed in these cases the cultures should be taken first as rose bengal is toxic to HSV and hence may affect the yield of the cultures as well as the PCR testing.

Marginal Keratitis

HSV marginal ulcer is an uncommon manifestation of viral reactivation and is often confused with staphylococcal marginal disease (Fig. 9.4). It is classically a dendritic ulcer present marginally, generally near a blood vessel and is associated with stromal infiltrate adjacent to an area of limbal injection (Fig. 9.5). It should be differentiated from a staphylococcal marginal ulceration.

HSV marginal ulcer begins as an ulcer and then is associated with an infiltrate whereas a staphylococcal ulcer begins as an infiltrate with intact epithelium and subsequently ulcerates (Table 9.2). With HSV infection there is generally limbal injection which can result in neovascularization whereas in staphylococcal marginal keratitis there is a lucid interval between the limbus and the ulcer. HSV generally progresses centrally where it may evolve into a dendrite whereas the staphylococcal
Viral Keratitis

Ulcer progresses circumferentially. Blepharitis is more often associated with staphylococcal infection and this occurs classically at 2, 4, 8 and 10 O’clock where the lids are in contact with cornea unlike HSV ulceration which can occur anywhere.

Sequelae of Infectious Epithelial Keratitis

Patients with infectious epithelial keratitis may undergo one of the four sequelae: A complete resolution of a dendritic or geographic ulcer may occur without any tell tale signs of previous epithelial keratitis. The patients may have dendritic epitheliopathy that is heaping of epithelium on the dendrite which is healing without any area of ulceration. The subepithelial branching pattern of HSV may occur as “ghost patterns” or the dendritic ulceration (Fig. 9.6) may progress to stromal keratitis and leave a dense scar with corneal thinning.

Neurotrophic Epithelial Keratitis

Neurotrophic keratopathy develops in patients with previous HSV epithelial disease. It occurs due to impaired corneal innervation and decreased tear formation, exacerbated by chronic use of topical medications, especially antiviral agents. Numerous epithelial or stromal recurrences or a prolonged and refractory epithelial infection may decrease corneal sensitivity and damage the basement membrane of the corneal epithelium. This results in recurrent epithelial erosions, and persistent non-healing sterile ulceration known as neurotrophic ulceration. These are also called trophic, indolent or metaherpetic ulceration. They are round or oval ulcers with grey, thickened and rolled up margins (Fig. 9.7). These ulcers may look similar to the infectious amoeboid herpetic ulcers and should be differentiated from them as the treatment of these two entities are different (Table 9.3).

Complications of neurotrophic keratitis include scarring, neovascularization, necrosis, perforation and secondary bacterial infection (Fig. 9.8).

---

<table>
<thead>
<tr>
<th>TABLE 9.2</th>
<th>Differentiation between HSV marginal keratitis and staphylococcal marginal keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>HSV marginal keratitis</td>
</tr>
<tr>
<td>Etiology</td>
<td>Infective</td>
</tr>
<tr>
<td>Epithelial defect</td>
<td>Present</td>
</tr>
<tr>
<td>Progression</td>
<td>Centrally</td>
</tr>
<tr>
<td>Limbal injection</td>
<td>More</td>
</tr>
<tr>
<td>Lucid interval between limbus and ulcer</td>
<td>Absent</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>Present</td>
</tr>
<tr>
<td>Blepharitis</td>
<td>Absent</td>
</tr>
<tr>
<td>Location</td>
<td>Any meridian</td>
</tr>
</tbody>
</table>

---

Figure 9.6: Healing herpetic epithelial keratitis (Courtesy: Medical Photographic Imaging Centre. Royal Victorian Eye and Ear Hospital, Melbourne)

Figure 9.7: Metaherpetic keratitis
Types of Microbial Keratitis

**HERPES SIMPLEX STROMAL KERATITIS**

Stromal involvement in primary cases is reported in 2 percent cases whereas it is responsible for 20 to 48 percent cases of recurrent ocular herpes. Stromal inflammation may occur primarily or may occur as a consequence of the epithelial infectious keratitis or endothelitis.

The two manifestations of herpes simplex virus stromal disease include the immune stromal keratitis and necrotizing stromal keratitis. It may also present as limbal vasculitis, immune ring of Wessely, necrotic interstitial keratitis, disciform keratitis and endothelitis with or without trabeculitis.

In stromal keratitis the patient presents with blurred vision and eye pain.

**TABLE 9.3**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Neurotrophic ulcer</th>
<th>Infectious ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of recurrent epithelial infections</td>
<td>Always positive</td>
<td>May be positive</td>
</tr>
<tr>
<td>Duration of ulcer</td>
<td>Long duration (indolent)</td>
<td>Relatively short</td>
</tr>
<tr>
<td>Location of ulcer</td>
<td>Interpalpebral area</td>
<td>Anywhere</td>
</tr>
<tr>
<td>Size of ulcer</td>
<td>Almost constant over a period of time</td>
<td>Increasing or decreasing depending on the success of antiviral treatment</td>
</tr>
<tr>
<td>Margins of ulcer</td>
<td>Shallow, clean</td>
<td>Raised, greyish and has devitalized virus laden epithelial cells</td>
</tr>
<tr>
<td>Rose bengal stain</td>
<td>Margins are not stained</td>
<td>Margins are heavily stained</td>
</tr>
<tr>
<td>Antiviral therapy</td>
<td>May make it worse</td>
<td>Promotes resolution</td>
</tr>
</tbody>
</table>

**Immune Mediated Stromal Keratitis**

Immune mediated stromal keratitis is also called interstitial keratitis and is the most common form of herpetic stromal disease. It is mediated by type III immune reaction to herpes simplex viral antigen and is associated with the deposition of antigen-antibody complex in the corneal stroma.

Clinically the disease is characterized by occurrence of stromal infiltration, neovascularization, thinning of the cornea, and recurrent inflammation. The overlying epithelium is always intact except in cases where epithelial ulceration leads to stromal involvement. However, corneal necrosis and ulceration are not seen. Secondary lipid keratopathy may occur as a result of persistent and recurrent inflammation.

Immune mediated stromal keratitis may manifest as one of the following:

**Punctate Stromal Opacities**

Punctate stromal opacities may be present which are associated with haze. They may be focal, diffuse or multifocal and may also be associated with anterior chamber inflammation.

**Immune Ring of Wessely**

It is a distinct clinical entity and is a type of immune mediated herpetic stromal keratitis. There is a circular deposition of antigen-antibody complex with polymorphonuclear leukocyte infiltration. It may be complete or incomplete and is present in the mid-stroma of central or paracentral cornea. Sometimes there may be two rings of stromal infiltrates called as double immune ring of Wessely (Fig. 9.9).
**Stromal Neovascularization**

Stromal neovascularization may occur acutely or in a chronic manner. It may be sectoral (Fig. 9.10) or may occur circumferentially (Fig. 9.11) when it occurs in a chronic manner. Aggressive therapy may cause the formation of "ghost vessels" which are empty channels of blood vessels devoid of any blood. Ghost vessels do not cause a diminution of vision or increase the chances of graft rejection subsequent to a penetrating keratoplasty.

**Necrotizing Stromal Keratitis**

Necrotizing stromal keratitis is a less common presentation of herpetic stromal disease. It is thought to be caused by direct invasion of HSV in corneal stroma, active viral replication and intense immune stromal inflammation. It generally occurs when topical corticosteroids have been given without the antiviral coverage.

Corneal necrosis, ulceration and dense infiltration of stroma with an overlying epithelial defect are the classical clinical features of this disease (Fig. 9.12). Secondary complications include hypopyon, uveitis, posterior synechiae, glaucoma, retrocorneal membrane, cataract and perforation.

**HERPETIC ENDOTHELITIS**

Herpetic endothelitis is caused by a delayed hypersensitivity reaction (Cell mediated type IV immune
Types of Microbial Keratitis

reaction) to herpes simplex viral antigen mediated by T lymphocytes. Endothelitis may present as disciform keratitis, diffuse endothelitis and linear endothelitis depending on the location of the KPs and the presence of overlying edema (Fig. 9.13).

Disciform Keratitis

The patient usually presents with symptoms of watering, photophobia, discomfort and blurred vision. It is the most common form of endothelitis in which disk shaped area of stromal edema overlying few KPs occurs without any corneal infiltration or vascularization. The area of involvement may be diffuse and central or eccentric. Disciform keratitis can occur without previous occurrence of herpetic corneal epithelial disease. Disciform keratitis may resolve without significant scarring (Fig. 9.14) or more severe attacks may develop into interstitial keratitis with stromal thinning and stromal necrosis. Perforation may occur if it is treated with steroids without antiviral cover. It may also be associated with trabeculitis and uveitis.

Diffuse Endothelitis

It is a rare presentation of ocular herpes simplex keratitis. Patients characteristically have scattered KPs over the entire cornea with overlying diffuse stromal edema and associated iritis (Fig. 9.15). It is an immune reaction targeted against the corneal endothelium. Aggressive treatment with topical corticosteroids lead to complete resolution of inflammation and edema.

Linear Endothelitis

It clinically appears as a line of KPs on corneal endothelium that progresses centrally from the limbus. It is accompanied by peripheral stromal and epithelial edema between the KPs and limbus.

HERPES SIMPLEX TRABECULITIS

Herpetic peripheral corneal involvement may extend to trabecular meshwork and cause trabeculitis and secondary glaucoma.

HERPES SIMPLEX IRIDOCYCLITIS

Herpes simplex keratitis may present as recurrent non-granulomatous anterior uveitis. The patient complains of pain, photophobia and ciliary flush. Iritis in a patient
Viral Keratitis

of previously known herpetic keratitis and should be considered herpetic until proved otherwise. It is thought to be an immunological reaction but live virus has also been demonstrated in some cases in the anterior chamber and in the iris. Anterior chamber inflammation is present and there may be iris atrophy, posterior synechiae and even iris hemorrhages.

**Laboratory Diagnosis**

The diagnosis of ocular herpetic disease, primary or recurrent is generally based on clinical examination. However in cases of atypical presentations or in cases where confirmation of diagnosis is required, laboratory diagnosis may be required.

**GIEMSA STAINING**

Corneal scraping in suspected herpes simplex keratitis is typically reserved for problematic cases, such as necrotizing stromal keratitis. Cytologic examination of Giemsa or Wright-stained specimens reveals multinucleated giant cells and the more pathognomonic Cowdry type A inclusions that are multi-nucleated, epithelial cells with ballooning degeneration. However, this test is not very sensitive and if negative does not rule out herpetic infection. It was found to have a sensitivity of 57.1 percent and specificity of 85.9 percent.

**POLYMERASE CHAIN REACTION (PCR)**

PCR is another useful diagnostic tool with high sensitivity. The sensitivity is almost equal to viral culture and the results are obtained much earlier. Its specificity has been found to be 67.9 percent in one study.

It can detect HSV in tear film and in corneal scrapings. Rose bengal and lissamine green may inhibit detection of HSV virus by PCR and therefore samples for PCR should be taken before staining.

**VIRAL CULTURE**

It is a definitive method of diagnosis but it may take several days before the test becomes positive. The lesion is swabbed and placed in viral transport medium or into the viral monolayer tubes and sent directly to the laboratory where the carrier medium is inoculated into cultures and the inoculated cells monolayers incubated at 37°C.

A typical cytopathic effect is generally noticed in 2-4 days but may require 5-10 days. The recovery rate from acutely infected ulcer is 70 percent if the specimen is taken within 2-3 days of the appearance of the lesion and then decreases as the clinical findings become more prominent. The use of antiviral drugs may decrease the recovery rate to 40 percent even in early disease.

**IMMUNOLOGICAL TESTS**

These tests include immunofiltration test, latex agglutination test and the enzyme linked immunoabsorbent assay (ELISA) test.

ELISA test is the immunologically based tests which produces results within hours. This test has 85 percent sensitivity in detecting the virus.

**Serological Test to Measure Circulating Antibodies**

Serum antibody titers can be used to differentiate primary herpetic infection from first ocular occurrence of recurrent disease. It is done by drawing paired sera from the patient. First sample is taken within 5-7 days of onset of the symptoms and the second sample is taken 3-4 weeks later. Presence of low neutralizing antibodies in the first sample followed by four-fold rise in the second titer indicate primary infection, whereas presence of moderate titers in the first sample followed four-fold rise in the second sample indicates re-infection or reactivation of the disease. Antibody titers can fluctuate independently of the presence of disease, and low positive titers are nearly ubiquitous in the adult population due to the widespread nature of herpetic disease. Both false-positive and false-negative test results are common. A suspicious flare up of keratitis associated with high HSV titers, along with a lower titer obtained during a period of inactive disease, can be considered strongly suggestive of HSV as an etiologic agent.

**Medical Treatment**

The drugs which are used for treatment of herpes simplex keratitis consist of antiviral agents and corticosteroids. Antiviral therapy may have to be given topically or systemically depending on the stage of the disease. The antiviral agents used for the treatment of viral keratitis include topical agents such as acyclovir, idoxuridine (IDU), vidarabine and trifluridine, and oral drugs such as acyclovir, valacyclovir, and famcyclovir. These antivirals are virostatic and inhibit viral replication by interfering with protein synthesis (Table 9.4).
Types of Microbial Keratitis

Management of Specific Conditions

The etiopathogenesis and management strategies in various manifestations of viral keratitis is shown in Table 9.5.

**INFECTIOUS EPITHELIAL KERATITIS**

The treatment of the infectious epithelial keratitis consists of physical debridement, topical antiviral agents, cycloplegics and tear substitutes. Physical debridement of the infected epithelial cells should be done with cotton tipped applicator. Topical antiviral drugs in the form of 3 percent acyclovir ointment 5 times/day or 1 percent trifluridine solution 2 hourly when awake are given for the first week. In cases which are responsive to therapy, after a week of treatment, these drugs are tapered that is, acyclovir is given three times a day and trifluridine is given 5 times per day. These are then continued in these doses for the rest of the treatment which lasts for 21 days. These drugs are discontinued after 3 weeks of therapy even when the ulcer does not heal completely. Generally, infectious epithelial keratitis shows signs of healing by 2 weeks.

Physical debridement of the infected epithelial cells should be done with cotton tipped applicator. Topical antiviral drugs in the form of 3 percent acyclovir ointment 5 times/day or 1 percent trifluridine solution 2 hourly when awake are given for the first week. In cases which are responsive to therapy, after a week of treatment, these drugs are tapered that is, acyclovir is given three times a day and trifluridine is given 5 times per day. These are then continued in these doses for the rest of the treatment which lasts for 21 days. These drugs are discontinued after 3 weeks of therapy even when the ulcer does not heal completely. Generally, infectious epithelial keratitis shows signs of healing by 2 weeks.

Cycloplegics (2% Homatropine hydrobromide or 1% Cyclopentolate hydrochloride) are added when iritis is present. Topical antivirals are very epitheliotoxic and it is imperative to give concomitant preservative free artificial tears (Hydroxypropyl methylcellulose 0.3% eye drops) to keep the epithelium lubricated.

In certain cases, if the dendritic patterns still persist after 2 weeks of therapy, a careful assessment is required if it is a true ulceration or a dendriti form epitheliopathy. The latter is a healing response of epithelium and is there is absence of any ulceration.

Corticosteroid drops are not recommended in the management of infectious epithelial keratitis unless significant stromal involvement coexists. In the event of coexisting stromal disease topical 3 percent acyclovir eye ointment is applied 5 times a day for 4-5 days to decrease the viral load and then topical corticosteroids (1% prednisolone acetate ophthalmic suspension) is started at a frequency of 2 to 4 hourly depending on the severity of stromal disease. After a week both the antiviral and corticosteroid are tapered very gradually over a period of 6 to 8 weeks. Sometimes tapering should be done over a longer period of time as the stromal disease has a tendency to recur immediately after the topical corticosteroids are stopped.

At times infectious epithelial keratitis may not respond to the topical antiviral medications. It is mandatory to re-scrape and send them for cultures as *Acanthamoeba* may be isolated in 70% of these cases.

Resistance generally does not occur in cases of viral keratitis; however if it occurs, and the patient is on acyclovir, vidarabine may be tried instead of acyclovir; trifluridine in these cases should not be used instead as trifluridine and acyclovir work by the same mechanism.

**NEUROTROPHIC KERATOPATHY**

Treatment is aimed at protecting the damaged basement membrane. Therapeutic approaches include stopping topical antiviral agents which may be epitheliotoxic and using preservative free artificial tears and ointment. In case the epithelium becomes boggy gentle debridement should be done. In cases of non-healing ulcers therapeutic bandage contact lenses may be given along with topical antibiotics to prevent contact lens induced microbial keratitis. Alternatively, a surgical tarsorrhaphy may be done or botulinum toxin injection may be given to cause a temporary lid closure. In severe cases, conjunctival flaps may be undertaken or amniotic membrane transplantation may be done (Figs 9.16A to C).

---

**Table 9.5**

<table>
<thead>
<tr>
<th>Antiviral agent</th>
<th>Route</th>
<th>Strength frequency</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidarabine (Ara-A)</td>
<td>Topical</td>
<td>3% ointment</td>
<td>Five times a day, inhibits viral DNA polymerase</td>
</tr>
<tr>
<td>Trifluridine</td>
<td>Topical</td>
<td>1% solution</td>
<td>Two hourly while awake, inhibits viral thymidylate synthase</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Topical, Oral</td>
<td>3% ointment 200,400 mg tablets</td>
<td>Five times a day, activated by viral thymidine kinase to inhibit DNA polymerase</td>
</tr>
<tr>
<td>Valacyclovir</td>
<td>Oral</td>
<td>1000 mg</td>
<td>Three times a day, activated by viral thymidine kinase to inhibit DNA polymerase</td>
</tr>
</tbody>
</table>
IMMUNOLOGIC DISEASE

A combination of antiviral drug and topical steroids is the recommended line of treatment of immune mediated HSV keratitis such as immune stromal keratitis and necrotizing stromal keratitis.

Immune Stromal Keratitis

Topical steroids are the mainstay for cases of immune stromal keratitis. Topical steroids reduce the severity and of stromal inflammation, decrease the duration of stromal keratitis and reduce corneal scarring, neovascularization and posterior synechiae formation.

Topical corticosteroids (1% Prednisolone acetate ophthalmic suspension, 4 times a day) should be started in mild cases. In more severe cases topical corticosteroids should be instilled every hour. In all the cases administration of topical corticosteroids should be accompanied with instillation of antiviral drugs (1% trifluridine solution or 3% acyclovir ointment).

A mydriatic–cycloplegic drug (2% homatropine hydrobromide or 1% cyclopentolate hydrochloride) added to reduce ciliary spasm.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Etiopathogenesis</th>
<th>Topical treatment</th>
<th>Systemic treatment</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic keratitis</td>
<td>Infection by live herpes simplex virus</td>
<td>3% topical acyclovir ointment (5 times/day) or 1% trifluridine solution (2 hourly) Epithelial debridement ±</td>
<td>None</td>
<td>Heals without significant corneal scarring</td>
</tr>
<tr>
<td>Amoeboid/ geographical ulcer</td>
<td>Infection by live herpes simplex virus</td>
<td>3% topical acyclovir ointment (5 times/day) or 1% trifluridine solution (2 hourly) Epithelial debridement ±</td>
<td>None</td>
<td>Heals without significant corneal scarring</td>
</tr>
<tr>
<td>Neurotrophic keratitis</td>
<td>Noninfectious, ↓ corneal sensitivity</td>
<td>Lubricants, BCL, patching, tarsorrhaphy, amniotic membrane</td>
<td>Collagenase inhibitors like tetracycline</td>
<td>Low dose topical corticosteroids and topical antiviral if stromal edema and inflammation present</td>
</tr>
<tr>
<td>Immune mediated stromal keratitis</td>
<td>Immune reaction to herpes virus antigen</td>
<td>Topical antivirals and topical corticosteroids</td>
<td>None</td>
<td>Very gradual tapering of topical therapy</td>
</tr>
<tr>
<td>Necrotizing stromal keratitis</td>
<td>Active viral replication in corneal stroma</td>
<td>Topical corticosteroids (1% prednisolone acetate suspension, 4 times a day) with 3% topical acyclovir ointment (5 times/day) or 1% trifluridine solution (9 times/day) ± mydriatic – cycloplegic drug (2% Homatropine hydrobromide or 1% cyclopentolate hydrochloride)</td>
<td></td>
<td>Gradual tapering off topical therapy</td>
</tr>
<tr>
<td>Endothelitis/ Disciform keratitis</td>
<td>Immune reaction to viral antigen</td>
<td>Topical corticosteroids (1% Prednisolone acetate ophthalmic suspension, 4 times a day) with 3% topical acyclovir ointment (5 times/day) or 1% trifluridine ± mydriatic – cycloplegic drug (2% homatropine hydrobromide or 1% cyclopentolate hydrochloride) solution (2 hourly)</td>
<td></td>
<td>Gradual tapering off topical therapy</td>
</tr>
</tbody>
</table>
Types of Microbial Keratitis

Necrotizing Stromal Keratitis

Topical corticosteroids (1% Prednisolone acetate ophthalmic suspension, 4 times a day) should be started in mild cases. In more severe cases topical corticosteroids should be instilled every hour. In all the cases administration of topical corticosteroids should be accompanied with instillation of antiviral drugs (1% trifluridine solution or 3% acyclovir ointment).

A mydriatic–cycloplegic drug (2% homatropine hydrobromide or 1% cyclopentolate hydrochloride) added to reduce ciliary spasm.

Topical corticosteroids should be tapered very gradually over months (more than 10 weeks) to avoid reactivation and rebound inflammation. In some patients topical corticosteroids cannot be tapered off without reactivation of the disease and in such cases low dose topical corticosteroids (0.01% dexamethasone ophthalmic solution or 0.125% prednisolone acetate ophthalmic suspension, 1 drop every day or every alternate day) should be continued indefinitely.

IRIDOCYCLITIS AND TRABECULITIS

It is mainly an immune reaction to viral antigen and in some cases to intact virus. The Herpetic Eye Disease Study (HEDS) found a trend toward improved response of herpetic iridocyclitis and trabeculitis when oral acyclovir was added to topical trifluridine and corticosteroids. A combination of oral antivirals and topical corticosteroids may provide sufficient antiviral coverage to allow for the desired suppression of immune response.

Patients are treated with combination of topical corticosteroids (1% Prednisolone acetate suspension, 4 times a day) and antiviral drugs (1% trifluridine solution or 3% acyclovir ointment) along with cycloplegics (2% homatropine hydrobromide or 1% cyclopentolate hydrochloride). Beta blocker (0.5% timolol maleate 2 times/day) and carbonic anhydrase inhibitor should be added to the above treatment if there is secondary glaucoma. Oral acyclovir 200 mg 5 times a day may be added in cases of severe uveitis or if live virus is isolated from the anterior chamber.

Recently, the efficacy of topical cyclosporine 0.05 percent (Restasis) in patients with herpes simplex virus non-necrotizing stromal keratitis unresponsive to topical prednisolone has been studied. It was found that herpes simplex virus stromal keratitis can be treated effectively with topical cyclosporine.

Surgical Treatment

The surgical therapies which need to be performed in severe cases of herpetic keratitis may include conjunctival flap, amniotic membrane transplantation, tarsorrhaphy, application of cyanoacrylate glue and a lamellar or penetrating keratoplasty (PKP).

Surgical treatment is required most often in cases of necrotizing stromal keratitis. In cases of recurrent herpetic keratitis with no potential vision, a gunderson flap may be done.

Penetrating keratoplasty following herpetic disease is accompanied by an increased risk of graft failure and recurrence of herpetic disease.

Complications after transplantation in herpetic eyes include corneal graft rejection, as well as herpes recurrences within the graft, persistent epithelial defect, corneal melting, secondary infection and graft failure. When PKP is performed for herpetic scar the risk of recurrence of herpetic infection is 12 to 19 percent. There are chances of occurrence of herpetic keratitis in eyes after PKP, even in patients with no previous history of HSV infection.
PREVENTION

The HEDS study has established that long-term (one year) oral antiviral therapy, with a dose of acyclovir 400 mg twice a day, can reduce the recurrence of epithelial keratitis and stromal keratitis. The benefit of such therapy was found to be greatest among patients with previous stromal keratitis, but in all groups benefits were found even if the previous episode of eye disease had occurred up to a year before therapy. For patients with epithelial keratitis only, the decision to begin prophylactic therapy must be made on an individual basis, but typically such prophylaxis is reserved for patients with two or more episodes in a year. In the treatment of epithelial keratitis, the HEDS study group did not find a benefit to adding 3 weeks of intensive oral acyclovir therapy to topical trifluridine.

Patients with any previous herpes simplex keratopathy who are undergoing PK may benefit from both preoperative and postoperative prophylaxis, and we do this routinely. Long-term oral acyclovir use seems to remain effective in decreasing the number of ocular herpes simplex virus recurrences. Similar prophylaxis is much more important for patients with suspected herpetic eye disease undergoing PRK or LASIK, since the short-wavelength laser can reactivate herpetic keratitis and lead to significant morbidity.

Oral valacyclovir 1000 mg three times a day has also found to be useful in prevention of herpetic keratitis.

Varicella Zoster Virus

Varicella zoster virus (VZV) belongs to the herpes virus family and cause varicella that is the chickenpox and the herpes zoster that is the shingles (Fig. 9.19). Varicella zoster virus causes a benign exanthematous illness manifested by prodromal symptoms and vesicular rash.
After acute infection the VZV travels down the peripheral axons to cells in the dorsal root ganglion where it remains in the latent phase.

The herpes zoster occurs due to reactivation of the VZV within the dorsal root ganglion and virions travel to skin or mucous membrane or skin along the axonal transport.

**EPIDEMIOLOGY**

The annual incidence of herpes zoster is 1.5/1000 to 3.0/1000 cases. The incidence is more in patients more than 75 years of age. There is no predilection for gender, race, or seasonal variation.

Herpes zoster has a greater preponderance in cases with altered cell mediated immunity, those taking immunosuppressive agents, organ transplant recipients, syphilis, tuberculosis, and those having HIV. It is also precipitated by physical or emotional stress.

The varicella zoster keratitis occurs in two forms:
- Primary (varicella)
- Recurrent (herpes zoster).

The ocular manifestations are uncommon in varicella but common in ophthalmic zoster. The various ocular manifestations in ophthalmic VZV include:
- Eye lesions which are manifested as pocks on lids and lid margins.
- Keratitis occurs rarely in cases of VZV.
- Epithelial keratitis with or without pseudodendrites occurs more rarely.
- Disciform keratitis with uveitis of varying duration can occur.

Ophthalmic varicella zoster infection is accompanied by keratouveitis that varies in severity according to immune status of the patient. The manifestations of ophthalmic varicella zoster infection are benign in children as compared to adults who have severe and sometimes blinding disease. Corneal complications in ophthalmic zoster are associated with skin eruption in areas supplied by branches of the nasociliary nerve.

Differences between dendrites of HSV and HZV infections (Table 9.6).

**TREATMENT OF OPHTHALMIC VARICELLA ZOSTER**

- Intravenous and oral acyclovir has been used successfully for treatment of herpes zoster ophthalmicus. This treatment regime is particularly important in immunocompromised patients. The appropriate timing of the therapy is vital. Therapy needs to be started within 72 hours after appearance of the rash. The use of oral acyclovir in a dosage of 800 mg five times daily for 10-14 days has been recommended. Although varicella zoster virus keratopathy is an uncommon indication for penetrating keratoplasty, effective visual rehabilitation can be achieved in these patients. Careful postoperative management, frequent lubrication, and lateral tarsorrhaphies to protect the corneal surface are major factors in the successful outcome of these cases.

**ADENOVIRAL INFECTIONS**

It is causative agent for epidemic keratoconjunctivitis which is predominantly caused by the serotypes 8, 19, and 37. The infection is highly contagious, with approximately 10 percent transmission in household contacts via hands and fomites. Transmission has also been associated with instrumentation, industrial trauma (shipyard workers (i.e. shipyard eye)), contaminated ophthalmic solutions, and the hands of health care workers. Corneal trauma facilitates infection. After an 8 days incubation period, an insidious onset of unilateral red eye occurs, which spreads to involve both eyes. Patients have photophobia, tearing, and pain (indicating corneal involvement). Children may have fever and lymphadenopathy. Malaise and headache are reported. Inflammation may persist for weeks, and residual scarring and visual impairment may occur. The associated findings in epidemic keratoconjunctivitis are:
- Severe follicular keratoconjunctivitis.

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<table>
<thead>
<tr>
<th>TABLE 9.6</th>
<th>Differentiation between dendrites of herpes simplex and zoster</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feature</strong></td>
<td><strong>HSV</strong></td>
</tr>
<tr>
<td>Overall</td>
<td>Fine, lacy</td>
</tr>
<tr>
<td>Epithelium</td>
<td>Linear defect with bared stroma, surrounded by edematous epithelial cells</td>
</tr>
<tr>
<td>Staining</td>
<td>Base stains with fluorescein</td>
</tr>
<tr>
<td>Disease border epithelial cells stain with rose bengal</td>
<td>None</td>
</tr>
<tr>
<td>Terminal bulbs</td>
<td>Frequent</td>
</tr>
</tbody>
</table>
Viral Keratitis

- Palpebral edema.
- Preauricular lymphadenopathy is not common but is a pathognomonic finding with adenovirus infection.
- Hemorrhagic conjunctivitis may develop.

In stage I corneal epithelial vesicle like elevations are present which are 25 to 30 microns and barely perceptible on slit lamp. Two to five days later, the lesions coalesce with each other, become clearly visible on slit lamp and involve deeper epithelium. These are the classical deep epithelial punctuate keratitis lesions which may resolve or progress further (Fig. 9.20). In stage III faint subepithelial infiltrates are present beside the deep punctate keratitis. Stage IV is characterized by nummular opacities which may be present months to weeks after the initial episode (Fig. 9.21).

Visual haziness or impairment resulting from keratitis develops due to the occurrence of nummular opacities (Fig. 9.21) and may persist for months to years.

A molecular assay for detection of human adenovirus based on automated nucleic acid extraction and real time polymerase chain reaction is being evaluated for the diagnosis of ocular adenoviral infections. The new molecular assay is suitable for rapid diagnosis of adenoviral keratoconjunctivitis in the routine diagnostic laboratory. It allows for a rapid diagnosis of adenoviral keratoconjunctivitis.26

Management

Medical management can range from cold compresses and artificial tears to topical vasoconstrictors (e.g. naphazoline) and steroids (vexol, flarex, pred forte) two to four times daily. Recently, cidofovir an antiviral drug used intravenously to treat cytomegalovirus retinitis appears to be effective in adenoviral keratoconjunctivitis. The topical form creates a faulty viral DNA structure. Twice daily instillation is recommended.27

References

Types of Microbial Keratitis

Keratitis can occur due to various protozoa, of which *Acanthamoeba* is the most notorious. Other protozoa which has been increasingly isolated from cases of corneal ulcer includes *Microsporidia*.

**Acanthamoeba Keratitis**

It was first described in the early 1970s, and a dramatic increase in cases of *Acanthamoeba* keratitis has been observed in the early to mid-1980s.

**Etiology**

The pathogenic species of *Acanthamoeba* include *Acanthamoeba castellanii*, *Acanthamoeba polyphaga*, *Acanthamoeba culbertsoni*, *Acanthamoeba palestinensis*, *Acanthamoeba astronyxis*, *Acanthamoeba hatchetti*, *Acanthamoeba rhysodes*, *Acanthamoeba divionesis*, *Acanthamoeba quna*, *Acanthamoeba lugdunensis*, and *Acanthamoeba griffini*.

The life cycle of *Acanthamoeba* consists of 2 stages: an active stage that is the trophozoite (which is 14-40 microns in diameter) stage and a cyst stage (which has a double-layered wall with a diameter of 12-16 microns) which is the dormant phase (Fig. 10.1).

**Epidemiology**

The incidence of *Acanthamoeba* keratitis varies depending on the geographic region and the type of contact lens use. In the UK, Europe and Hong Kong, the rate of incidence of *Acanthamoeba* keratitis was estimated to be 0.33 per 10,000 in hydrogel contact lens wearers per year.\(^1\) In the recent years an increased incidence of new cases of *Acanthamoeba* has been noted from USA.\(^2\) The incidence of *Acanthamoeba* keratitis associated with use of rigid contact lenses is 9.5 times lower than for soft lens wearers.\(^1\)

The disease is generally unilateral; however bilateral cases have also been reported. There is no sex predilection.

**Risk Factors**

There are various risk factors which predispose to *Acanthamoeba* keratitis (Table 10.1).\(^3-5\) The majority of the cases occur in contact lens wearers.\(^5\) However, in the Indian subcontinent it has also been reported in non-contact lens wearers.\(^6\) Apart from this there may be other risk factors which are associated apart from this in the

**Table 10.1**

**Risk factors for *Acanthamoeba* keratitis**

- Contact lens wearers
- Contaminated water/solutions especially home made solutions
- Corneal trauma
- Orthokeratology
Types of Microbial Keratitis

Indian subcontinent including patients who have undergone orthokeratology.

Contact Lens Wear

Classically, it occurs in immunocompetent, healthy, young individuals and the most important factor in these cases is contact lens wear which occurs in 80 to 86 percent of these cases.\(^3\)\(^5\) No type of contact lens has been excluded from an association with *Acanthamoeba* keratitis. Out of the 75 percent of the patients who are contact lens wearers, 40 percent were soft contact lens wearers, 22 percent were rigid gas permeable wearers and 38 percent were either extended wear contact lens wearers or other types of contact lenses.\(^7\)

The safest form of contact lens wear remains daily disposable lenses as very few cases of *Acanthamoeba* keratitis have been reported in patients using daily disposable lenses.\(^2\)

The first generation of silicone hydrogel contact lenses which used balafilcon A material showed increased chances of adherence of trophozoites\(^8\) as compared to the second generation silicone hydrogel contact lenses which use galyfilcon A.\(^9\)

It is to be noted that most commercially available contact lens disinfection solutions are ineffective against *Acanthamoeba*.\(^10\)

Non-Contact Lens Wearer

In India *Acanthamoeba* keratitis has also been reported in cases of non-contact lens wearers\(^6\) and the major risk factor for *Acanthamoeba* keratitis in these cases was corneal trauma.

Orthokeratology

Orthokeratology, that is the use of contact lens wear to shape the cornea has also been associated with *Acanthamoeba* keratitis the incidence of which was found to be as high as 30 percent out of all pathogens.\(^11\)

Other Risk Factors

Other risk factors associated with *Acanthamoeba* keratitis include use of contaminated water or solutions including the home made solution to disinfect contact lenses, swimming with contact lenses on and corneal trauma.

**SYMPTOMS**

The major clinical symptoms are severe or moderate pain, decreased vision, redness, irritation, foreign body sensation, photophobia, mucous discharge and tearing (Table 10.2). One of the most important symptoms of *Acanthamoeba* keratitis is severe pain especially in the early phase of the infection.

Classically *Acanthamoeba* keratitis follows a protracted waxing and waning course. The phase of remission reflects the presence of dormant cysts, whereas the progression of keratitis is due to the emergence of replicating trophozoites from the cysts.

**SIGNS**

*Acanthamoeba* infection classically involves the cornea. However, in some cases lids, anterior chamber and sclera may also show signs of inflammation (Table 10.2).

**CORNEAL INVOLVEMENT**

Early corneal signs included epithelial stippling with microcystic edema; coarse, opaque streaks; fine epithelial and subepithelial curvilinear opacities; and dendritiform epithelial lesions (Fig. 10.2).

<table>
<thead>
<tr>
<th>Table 10.2</th>
<th>Clinical features of acanthamoeba keratitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms—waxing and waning course</strong></td>
<td></td>
</tr>
<tr>
<td>• Severe pain/foreign body sensation</td>
<td></td>
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<tr>
<td>• Redness</td>
<td></td>
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<tr>
<td>• Watering, mucus discharge</td>
<td></td>
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<tr>
<td>• Decreased vision</td>
<td></td>
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<td>• Photophobia</td>
<td></td>
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<tr>
<td><strong>Signs—corneal</strong></td>
<td></td>
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<tr>
<td>• Corneal epithelial abnormalities</td>
<td></td>
</tr>
<tr>
<td>• Epithelial haze</td>
<td></td>
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<tr>
<td>• Elevated lines</td>
<td></td>
</tr>
<tr>
<td>• Microcysts</td>
<td></td>
</tr>
<tr>
<td>• Pseudodendrites</td>
<td></td>
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<tr>
<td>• Punctate epithelial erosions</td>
<td></td>
</tr>
<tr>
<td>• Stromal infiltrates</td>
<td></td>
</tr>
<tr>
<td>• Radial keratoneuritis (Along nerves of anterior stroma)</td>
<td></td>
</tr>
<tr>
<td>• Ring shaped stromal infiltrate</td>
<td></td>
</tr>
<tr>
<td>• Stromal thinning and furrowing</td>
<td></td>
</tr>
<tr>
<td>• Satellite lesions</td>
<td></td>
</tr>
<tr>
<td>• Stromal ulceration and perforation (rare)</td>
<td></td>
</tr>
<tr>
<td><strong>Signs—others</strong></td>
<td></td>
</tr>
<tr>
<td>• Lid edema, pseudoptosis</td>
<td></td>
</tr>
<tr>
<td>• Conjunctival injection, chemosis</td>
<td></td>
</tr>
<tr>
<td>• Iritis, hypopyon</td>
<td></td>
</tr>
<tr>
<td>• Dacryoadenitis</td>
<td></td>
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<tr>
<td>• Reactive Ischemic Retinitis</td>
<td></td>
</tr>
<tr>
<td>• Secondary Glaucoma</td>
<td></td>
</tr>
<tr>
<td>• Cataract</td>
<td></td>
</tr>
<tr>
<td>• Severe anterior and posterior scleritis</td>
<td></td>
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</tbody>
</table>
Protozoal Keratitis

Radial keratoneuritis as a presenting sign in Acanthamoeba keratitis has been described by Moore et al. Radial keratoneuritis is characterized by linear radial, branching infiltration by the parasites along corneal nerves in the anterior stroma. This infiltration begins paracentrally and extends to the limbus in a radial pattern, and is responsible for the severe degree of pain (Fig. 10.3).

The late stromal infection is characterized by dense single or multiple stromal infiltrates which may be central or paracentral and a characteristic ring infiltrate. The overlying epithelium may remain intact and an intrastromal abscess may occur. However, stromal neovascularization is never seen even in severe and longstanding cases.

A ring-shaped stromal infiltrate is an indicator of advanced infection and is nearly pathognomonic for Acanthamoeba keratitis which may be present in 19 to 93 percent of cases. The infiltrate may be segmental or circumferential, progressive, and often involves stromal thinning (Fig. 10.4) or furrowing, and may be associated with a variable overlying epithelial defect. It is due to polymorphonuclear leukocyte infiltrates generated by chemotaxis after antigen-antibody precipitation due to collagenolytic enzymes released by Acanthamoeba which degrades collagen and produces ring infiltrates.

As the disease progresses, there may be progressive loss of corneal stroma with formation of descemetocele and perforation and this is more common when there is bacterial super-infection (Fig. 10.5).
Types of Microbial Keratitis

Lids and Conjunctiva Involvement
Other associated clinical signs include lid abnormalities, lid edema, reactive pseudoptosis and associated iritis. Conjunctival injection and chemosis are almost always present.

Iritis and Hypopyon
In severe cases there may be an associated iritis and hypopyon formation.14

Cataract and Glaucoma
Other complications of prolonged Acanthamoeba keratitis include cataract formation, with incidence ranging from 20 percent to slightly less than 40 percent.13,15 This may occur as a result of organism-specific inflammation and infection, or may be attributed to the chronic use of toxic topical antiamoeba agents.

Severe glaucoma is another complication in cases of prolonged Acanthamoeba keratitis, with a reported incidence of 30 percent, and may require drainage procedures to control the intraocular pressure.16

Scleral Involvement
Acanthamoeba keratitis may be associated with a prominent scleritis, which may be responsible for the boring, severe pain in the later stages of the disease. It is characterized by deep scleral vascular engorgement and scleral nodules, the frequency of which ranges from 11 to 42 percent depending on the stage of infection.13 Most patients have sectional areas of anterior scleritis contiguous with areas of keratitis (Fig. 10.6). In most cases, scleritis is a secondary immunologic response, rather than a true scleral infection of Acanthamoeba.13,14 Unexpectedly, prolonged ocular inflammation and infection with deep corneal abscesses have been recently shown to result in a new syndrome of retinal ischemia through severe reactive posterior segment inflammation.13

DIFFERENTIAL DIAGNOSIS
Acanthamoeba infection can mimic several other types of keratitis, including viral bacterial or fungal keratitis. Parmar et al recently showed that 26 (41%) of 63 patients confirmed to have Acanthamoeba keratitis by culture or confocal microscopy were initially diagnosed and treated as having herpes keratitis, whereas 43 percent were misdiagnosed as bacterial keratitis.17

Herpes Keratitis
Acanthamoeba keratitis is frequently misdiagnosed as herpes simplex keratitis. It has been reported that herpes keratitis was misdiagnosed in 70% cases of Acanthamoeba keratitis in one study.18 Minor trauma may be associated with both, that is, the herpes simplex keratitis and Acanthamoeba keratitis. However, trauma as a predisposing factor for Acanthamoeba keratitis is more common as there is also concomitant contamination with soil or water. Nevertheless, contact lens wear is not a frequent predisposing factor in herpes simplex keratitis.

Clinically, in early stages of infection with herpes simplex the dendritic lesion is ulcerated, whereas in Acanthamoeba keratitis, the pseudodendrite is elevated at the center of the cornea, and the epithelium has a gelatinous appearance. The stromal infiltrates in herpes simplex infection tend to be smaller as compared to Acanthamoeba infection. However, the more advanced stages of the disease, are accompanied by ulceration and loss of stromal tissue and the keratitis may resemble herpes simplex infection.

Fungal Keratitis
Acanthamoeba keratitis can also mimic fungal keratitis and this has been particularly reported from the Indian subcontinent.9 This may occur due to the presence of
stromal infiltrates and endothelial plaques. Severe pain, annular infiltrates and radial keratoneuritis are frequently absent in cases of fungal keratitis.

**Laboratory Diagnosis**

*Acanthamoeba* keratitis should be diagnosed as early as possible, because therapy is most effective when initiated early and requires prolonged treatment.

**CORNEAL SCRAPING**

Corneal scraping should be taken from the area of abnormal epithelium and ulceration. It may fail to reveal the presence of Acanthamoeba in early cases, when epithelium is intact or in cases in which superficial cornea is unaffected.

**WET SMEAR**

Fresh wet mount specimens can also be used. The use of a spray fixture to avoid trophozoite disruption by air drying has been recommended.

**STAINING**

Trophozoites and cysts can be identified in the corneal scrapings or smears by staining with hematoxylin and eosin, Gram (Fig. 10.7), Giemsa-Wright, Calcofluor, methylene blue, congo red, Janus green, Lugol solution, acridine orange or wheatly trichrome stains. Light microscopy of cysts and motile trophozoites is facilitated using Nomarski optics. The staining characters with various stains are described in Table 10.3.

A study performed on corneal histopathologic specimens showed that hematoxylin and eosin is adequate to highlight the *Acanthamoeba* trophozoites and cysts for an experienced opthalmic pathologist; followed by periodic acid Schiff staining. The latter two stains should be used routinely when screening for *Acanthamoeba* in corneal tissue, whereas Gomori methenamine silver should be done whenever there is appreciable inflammation in tissue structures, because the amoeba can sometimes resemble inflammatory cells (macrophages), and hematoxylin and eosin would then not be adequate to make the distinction.

The trophozoite is characterized by a large single nucleus and spindle like pseudopodia. It is easier to recognize the cysts, which are double walled, with the inner wall having a variety of polygonal shapes.

Calcofluor white is a relatively simple and reliable method for detection of *Acanthamoeba*, even in cases in which Gram and Giemsa fail to reveal organisms. Calcofluor white is a fabric brightener and has an affinity for chitin and cellulose, which are components of cell walls of *Acanthamoeba* cysts and fungi but not *Acanthamoeba* trophozoites.

A solution containing 0.1 percent cell calcofluor white and 0.1 percent Evans blue counter stain is applied to the specimen for 5 minutes. The specimen is then examined under fluorescence microscope. *Acanthamoeba* cysts appear as apple green structures 10-25 μm in diameter. Trophozoites are considerably more difficult to detect with this method because they do not absorb calcofluor white. However, Evans blue counter stain shows the trophozoites as red brown, irregularly shaped structures 15 to 20 μm in length.
Types of Microbial Keratitis

Fluorescein–conjugated lectins such as Concanavalin A and wheat gram agglutinin stain both trophozoites and cyst. Under fluorescent microscopy, the cyst walls stain green and the trophozoites stain red. In addition, the more specialized technique of indirect fluorescent antibody staining is thought to be more sensitive and specific.

CULTURE MEDIA

The scraped material should be directly inoculated on a confluent lawn of Escherichia coli (monoaxonic culture) plated on non-nutrient agar. The laboratories have recommended a temperature of 35°C, possibly with a second plate at 30°C or even 25°C. The culture plates should be sealed with adhesive tape to prevent evaporation and loss of Acanthamoeba organisms from drying.

Organisms such as Escherichia coli, Aerobacter aerogenes, Enterobacter species, Klebsiella pneumoniae or Xanthomonas maltophilia are used as a source of nutrition for Acanthamoeba on nutrient agar medium. Acanthamoeba trophozoites track through the lawn of the bacteria. The bacteria do not fill in these paths as there is absence of nutrition for bacteria in the non-nutrient agar. The path depicts the ingestion of bacteria by trophozoites; the bacteria are unable to reproduce fast enough to fill in the defect in the nutrient poor medium. Large numbers of trophozoites are usually seen by 3 days of incubation but may appear as early as 1 day.

At least one serial transfer should be done to confirm the amoebic isolation, as both macrophages and polymorphonuclear leukocytes can produce “pseudotrials” on primary isolation. Macrophages and polymorphonuclear cells become non viable rapidly, and on serial transfer do not form trails.

The cultures may require more than 9 days to recover the organism and should be maintained for more than 2 weeks. The success rate of culturing Acanthamoeba from corneal scrapings is 44-74 percent.

Method for Examining Contact Lens

An excised fragment of the contact lens is placed on a microscope slide with a cover slip and examined unstained or after staining with 0.1 percent Calcofluor White. Evans blue counter stain should not be used as it stains the contact lens intensely. The anterior and posterior surfaces of the lens are examined with X200 to X400 magnification for the presence of Acanthamoeba.

A fragment of the contact lens may be placed directly on the non-nutrient agar with an E.coli overlay. If Acanthamoeba are present, its trophozoites will migrate onto the culture plate.

For wet mount preparation contact lens solution is best centrifuged at 250 revolutions per minute and the sediment is transferred to a slide and covered with a cover slip. The slide should be kept in a covered Petri dish and can be examined using phase microscopy. Alternatively, large volumes of contact lens solutions can be filtered through a 5μm polycarbonate membrane filter, which is then placed upside down on the culture plate.

POLYMERASE CHAIN REACTION

Polymerase chain reaction testing may be applied on epithelial scrapings to improve the yield, but it is still experimental, appears to be highly strain-specific, and requires the use of numerous screening probes.

CORNEAL BIOPSY

A corneal biopsy should be considered if the epithelium is intact but the stromal lesion is active. It is particularly valuable in cases in which the infiltrate is deep in the cornea. A 1.5 to 2.0 mm corneal trephine may be used to obtain biopsy specimen from an area of infiltration peripheral to the visual axis.

Non-nutrient agar plates can be used to culture the organisms in biopsy specimens. Other staining procedures such as hematoxylin and eosin, periodic acid Schiff, methenamine silver, calcofluor white, or fluorescein labeled antibodies against Acanthamoeba can be used to stain organisms in biopsy specimens. Moreover, electron microscopy techniques can be applied to identify the parasite in the corneal tissues.

By contrast, corneal biopsy is performed masked and is limited by its anterior location and yield. It is believed that Acanthamoeba trophozoites and cysts are found in the anterior stroma initially and move deeper into the stroma with prolonged infection. Topical drugs will act on the more superficially positioned amoebas, whereas the deeper organisms, particularly in large confluent abscesses, are not susceptible to eradication by antiamoeba medications.

CONFOCAL MICROSCOPY

Confocal microscopy has been used in the diagnosis and management of Acanthamoeba keratitis because of the
ability to detect the organism in the cornea in vivo. This technique can also be used to monitor patients who have been treated for Acanthamoeba keratitis. It can also be used to assess if any cysts or trophozoites are present prior to transplantation surgery. One major advantage of this method is that it is essentially non-invasive, although it requires a cooperative patient and a skilled operator. In addition, it seems quite likely that this method is more sensitive in identifying Acanthamoeba organisms in early infections, which should improve outcomes in these cases. In more advanced cases, it also aids in differentiating epithelial drug toxicity from persistent disease. Using the confocal microscope, one can visualize high-contrast, real-time images of corneal sections at magnifications of x 240-380 on a video monitor.

The cystic form of Acanthamoeba is more distinct and appears as a double-walled, hexagonal, hyperreflective structure that is 10-25 microns in diameter (Fig. 10.5). The trophozoite form is more difficult to discern, as it appears similar to normal corneal keratocyte nuclei: an ovoid, S-shaped, structure within the corneal stroma. Other ovoid objects may be observed during confocal microscopy of patients with Acanthamoeba keratitis and may represent inflammatory cells, trophozoites and altered keratocytes. Confocal microscopy also helps to rule out any concomitant infection with fungal organisms.

The sensitivity of this test for the diagnosis of suspected Acanthamoeba keratitis in experienced hands has been reported to be 98 percent, whereas its specificity is yet to be determined. Such a powerful tool, however, requires a skilled operator, a qualified and experienced reader, and a compliant patient. It is also a relatively expensive technology available only in select referral centers.

OTHER TESTS

Although Acanthamoeba can be identified because of its cystic structure and acanthopodia, species identification is difficult. Morphologic characteristics of cyst stage and isoenzyme analysis have been used to identify different species of Acanthamoeba. More recently, restriction enzyme analysis of either mitochondrial DNA or cellular DNA were applied to differentiate species of parasites. However, these characteristics were not correlated with morphologic identification of different species.

TREATMENT

The treatment of Acanthamoeba keratitis is difficult, as the cystic form is highly resistant and may persist for years.

The drugs which have been found to be effective against Acanthamoeba include propamidine 0.1 percent (Brolene), neomycin 1 percent and cationic antiseptic agents such as chlorhexidine (0.02%) and polyhexamethylene biguanide (PHMB, 0.02%). However, most of these medications are not commercially available and must be obtained through compounding pharmacies.

Most clinicians advocate a combination therapy and treatment with PHMB or chlorhexidine which is generally given in combination with a diamidine, either propamidine (Brolene) or hexamidine (Desmodine).

COMBINATION THERAPY

Therapy is effective if definitive treatment starts within one month of onset. Combination therapy consists of use of two or three medications simultaneously. This includes use of topical PHMB (0.02%) or chlorhexidine (0.02% or 0.04%) without propamidine 0.1 percent. They should be given every hour around the clock for the initial 72 to 96 hours, 2 hourly for 2-4 weeks and then tapered to qid dose for 6-12 months.

Topical cycloplegic therapy and oral nonsteroidal drugs are helpful in the management of pain.

The later the treatment is started, the deeper the Acanthamoeba cysts are in the corneal stroma and it is more difficult to eradicate the large abscesses. Little data are available in the literature about the penetration of antiamoeba agents, but the deeper cysts, especially in large abscesses, are shielded from the cysticidal concentrations, necessitating longer exposure. This however may be associated with tissue toxicity from the treating agents. It has been found helpful to reduce antiamoeba eyedrops abruptly to four times a day and observe. If there is any reactivation, the eyedrops are increased to hourly or every 2 hours, and tapered slowly once again. This mode of treatment may catch the resistant cysts, especially the deeper ones, in the process of converting to the more drug-vulnerable trophozoites (pulse therapy).

According to Awwad et al, following the initial treatment, the clinical picture may worsen during the first weeks in terms of infiltrate density, stromal scarring, conjunctival injection, and pain, especially if topical
steroids or nonsteroidal agents have been used before diagnosis and are stopped abruptly. However, clinical worsening that develops after the first month can be due to the worsening of the infection or cumulative drug toxicity. In the absence of confocal microscopy, it may be hard to differentiate these two entities, which require totally different treatment approaches. The authors advise tapering the medications and evaluating closely. Subsequent improvement denotes drug toxicity, whereas further deterioration indicates an underlying intractable infection, which should be treated with more frequent regimen and possibly higher drug concentrations.

The possibility of a coinfection with bacteria or fungi should be constantly borne in mind, and repeat corneal culturing and confocal examination should be performed when in doubt.

Resistance to antiamoeba agents, such as chlorhexidine and PHMB, has not been documented, and treatment failure is due to lack of corneal penetration and failure to reach cysticidal concentration in deeper stroma in conditions in which the entire cornea may be involved. Hence, in vitro sensitivity testing is not routinely performed.

IMIDAZOLES

Topical imidazoles, when used in a 1 percent solution, are effective against trophozoites, but not against cysts. They should never be used as monotherapy. Oral ketoconazole and, to a lesser extent, itraconazole penetrate into the cornea and are used by some practitioners as adjunctive therapy to PHMB and chlorhexidine. The penetration of these drugs into cornea is inappropriate for them to act as trophozoiticidal agents and hence should not be used alone.

NEOMYCIN

Most cysts are resistant to neomycin which also has a high hypersensitivity rate and hence the use of neomycin is no longer recommended.

ROLE OF CORTICOSTEROIDS

Corticosteroids suppress the activity of the macrophage, which is essential in scavenging and destroying the amoeba. Thus, in general, it is recommended to delay and limit steroid use as much as possible. They are used in only those patients with severe pain, stromal lysis, scleritis, chronic ulcers or severe anterior-chamber inflammation under the cover of anti-amoebic drugs.

Systemic immunosuppression with steroids combined with antiamoeba therapy has also been described to control pain and tissue destruction for intractable *Acanthamoeba* sclerokeratitis. In one study, 20 eyes of 19 patients received systemic immunosuppression with steroids during a mean period of 7 months, with two eyes remaining in severe pain and necessitating enucleation.

SURGICAL TREATMENT

Various surgical procedures for *Acanthamoeba* keratitis which have been tried include penetrating keratoplasty, lamellar keratectomy with conjunctival flap and even amniotic membrane transplantation.

PENETRATING KERATOPLASTY

The results of therapeutic keratoplasty in eliminating the infection in *Acanthamoeba* keratitis is variable. A cure rate of 50 percent and a functional success rate of 50 percent have been reported. The rate of recurrence of the infection in the graft after therapeutic keratoplasty varies from 30-50 percent. Ficker et al reported a recurrence rate of more than 50 percent with poor graft survival. Due to a high recurrence rate of *Acanthamoeba* in therapeutic grafts medical control of infection has been recommended first. There should be a minimum gap of 3 months between the complete resolution of *Acanthamoeba* keratitis on medical therapy and optical penetrating keratoplasty.

Meticulous management and frequent follow up of cases after penetrating keratoplasty for up to 1 year is required as *Acanthamoeba* can recur in a graft.

LAMELLAR KERATOPLASTY

Lamellar keratoplasty with a conjunctival flap has been used successfully in some patients.

AMNIOTIC MEMBRANE TRANSPLANTATION

Amniotic membrane transplantation for progressive stromal lesions with persistent epithelial defects may also be effective in controlling inflammation and delaying penetrating keratoplasty with good success. It is sometimes necessary to repeat the amniotic membrane transplantation to ensure complete re-epithelialization.
References

11. Saviola JF. The current FDA view on overnight orthokeratology: how we got here and where we are going. Cornea 1997;16:770-1.
Specific Types of Keratitis

SECTION 4
Pediatric Keratitis

Introduction

Microbial keratitis is an important cause of ocular morbidity and blindness in children. Children and their families are frequently unable to provide a complete history of important risk factors such as previous trauma, duration of symptoms and contact lens cleaning regimes. Older children may give incomplete or false information because of fear of possible parental reprisals for circumstances surrounding the cause of keratitis. Poor co-operation may make the results of slit-lamp examination less reliable.

Epidemiology

PREVALENCE

In general children account for 11 percent of the cases according to a review of microbial keratitis in Southern California. Recent figures of World Health Organization suggest that there are 1.5 million children blind worldwide and that 70,000 children annually have active corneal involvement. It is of a greater concern in the developing world; 22 percent of 201 patients admitted at a referral center in South Africa were under 16 years of age.

Most studies have described microbial keratitis in the age group 16 years or younger. It is generally believed that corneal ulceration is not very common in children younger than 5 years. However, it has been reported in children younger than 5 years in one study from Northern India (Table 11.1).

There is no predilection for any eye and an almost equal number of right and left eye involvement has been noted (Table 11.1). Bilateral affections may also occur and have been attributed to systemic illness (Table 11.1).

REGIONAL DIFFERENCES

Just like the prevalence of different organisms responsible for adult microbial keratitis varies in different regions in the world, the organisms responsible for childhood keratitis also vary.

Gram-positive cocci are the commonest isolates in pediatric keratitis in Southern California (83%), New Orleans / Philadelphia (54.6%) and India 75% and 85.5 percent. However, in Florida gram-negative bacilli (43.2%) are the most prevalent.

In India, fungal infections were found to be relatively more (17.2%) as compared to California (4%). Filamentous fungi are particularly more in Indian subcontinent (14.1%) as compared to the Western world (2%).

Predisposing Factors

Risk factors for the occurrence of pediatric keratitis include trauma, severe systemic illness, contact lens wear and pre-existing external eye disease.

TRAUMA

Trauma is the leading predisposing factor of microbial keratitis regardless of age and has been reported in 24-44 percent cases (Table 11.2). The introduction of the organism is through an epithelial defect or gap in epithelial bridges or is concurrent with penetrating or perforating corneal injury.

SYSTEMIC FACTORS

Systemic illnesses associated with corneal ulceration vary from region to region. These include eruptive fevers such as measles, diarrhea and fever in India. There is a high prevalence of Vitamin A deficiency leading to
Specific Types of Keratitis

TABLE 11.1
Demographic features of microbial keratitis in children

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Author/ (Year)</th>
<th>No. of eyes/ No. of Children</th>
<th>Age group (%)</th>
<th>Male/Female Left eye (%)</th>
<th>Right eye (%)</th>
<th>Bilateral affection (%)</th>
<th>Seasonal pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ormerod et al(^4) (1986)</td>
<td>47/44</td>
<td>&lt;16 years</td>
<td>67/33</td>
<td>49/51</td>
<td>6.8</td>
<td>July to December</td>
</tr>
<tr>
<td>2.</td>
<td>Cruz et al(^10) (1993)</td>
<td>51/50</td>
<td>&lt;16 years</td>
<td>68/32</td>
<td>51/49</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>3.</td>
<td>Clinch et al(^5) (1994)</td>
<td>29/28</td>
<td>&lt;16 years</td>
<td>64/36</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4.</td>
<td>Kunimoto et al(^1) (1998)</td>
<td>113/107</td>
<td>&lt;16 years</td>
<td>56/44</td>
<td>51/49</td>
<td>5.6</td>
<td>May, June, November</td>
</tr>
<tr>
<td>5.</td>
<td>Vajpayee et al(^9) (1999)</td>
<td>50/50</td>
<td>&lt;12 years</td>
<td>68/32</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

TABLE 11.2
Predisposing factors for microbial keratitis in children

<table>
<thead>
<tr>
<th>Predisposing Factor (%)</th>
<th>Ormerod(^4)</th>
<th>Cruz(^10)</th>
<th>Clinch(^5)</th>
<th>Kunimoto(^1)</th>
<th>Vajpayee(^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trauma</td>
<td>29.7</td>
<td>44</td>
<td>34.5</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>Severe systemic illness</td>
<td>6.3</td>
<td>14</td>
<td>27.6</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Contact lens wear</td>
<td>6.4</td>
<td>12</td>
<td>24.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exposure</td>
<td>25.5</td>
<td>—</td>
<td>24.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ocular surgery</td>
<td>4.2</td>
<td>24</td>
<td>20.7</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Congenital anterior segment disease</td>
<td>27.6</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>Acquired anterior segment disease</td>
<td>6</td>
<td>20</td>
<td>12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Others</td>
<td>17</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

keratomalacia in Indian subcontinent (58.8% of systemic cases)\(^1\) and is especially important in cases with bilateral affection. Lower socio-economic status may be significantly associated with the occurrence of corneal ulceration.\(^9\)

Measles associated corneal ulceration is also an important cause of viral keratitis in the developing countries unlike the developed countries.\(^11\) In a study from Tanzania 25 percent of the unilateral cases and 57 percent of the bilateral corneal ulcers occurred within one month of the measles infection.\(^11\) Keratitis in measles may occur due to superadded herpetic infection. Ulcers associated with measles are varying from typical measles induced superficial keratitis to frank ulceration, which are generally epithelial and accentuated by desiccation due to exposure.\(^11\)

In the Western countries, systemic aspergillosis, bronchopulmonary dysplasia, Werdnig-Hoffmann’s disease, neurofibromatosis, hydrocephalous and mal-development of brain have been reported as the risk factors for the occurrence of pediatric microbial keratitis.\(^5\)

In a study by Ormerod et al, systemic infections and malignancies with orbital involvement were the main systemic associations of pediatric microbial keratitis.\(^4\)

CONTACT LENS WEAR

Contact lens wear as a pre-disposing factor for microbial keratitis has been reported in various western studies and varies from 6.4 to 24.1 percent.\(^4,5,10\) However, contact lens wear is not an important predisposing factor in India\(^1,5\) (Table 11.1). Contact lenses are not routinely prescribed in Indian subcontinent because of tropical environment and socioeconomic constraints.\(^9\)

Contact lenses predispose to keratitis especially in aphakic children. Orthokeratology is also an important risk factor for contact lens induced infection.

The extended wear lenses are more prone to contact keratitis especially if the contact lens hygiene and disinfection is not adhered to.
PRE-EXISTING EXTERNAL EYE DISEASE/SURGERY

Exposure keratitis, trichiasis, dry eye are important in infancy and trauma and acquired external disease are more common in the school age group. Other previous eye diseases include spring catarrh, dacryocystitis, and conjunctivitis.

Prior anterior segment surgery performed on the same eye has also been reported as a risk factor in 8.8 to 20.7 percent cases.

OTHERS

Use of traditional eye medicines is common in developing countries and has been shown to be associated with corneal ulceration in 14 percent cases in a study from Tanzania.

Etiology

MICROBIAL KERATITIS

Just like in adults, infectious microbial keratitis in children may be caused by bacteria, fungi, viruses or parasites.

Bacteria

Gram-positive cocci are the most frequently isolated organisms in cases of non-viral microbial keratitis in children (34-75%). However, in a series by Cruz et al more Gram-negative bacilli (43.2%) were identified as compared to the gram-positive cocci.

Staphylococcal species and Pseudomonas species have been isolated most commonly in cases of pediatric corneal ulceration [Table 11.3]. Indigenous bacteria such as non-coagulase positive Staphylococcus have also been increasingly reported in the recent times particularly from the Indian subcontinent. This occurs especially in eyes, which have already been compromised by trauma, previous eye disease or systemic illness. Functional and morphologic disturbances of the ocular surface in these eyes facilitate infection by indigenous bacteria.

Pseudomonas aeruginosa infections are more common in younger children as compared to older children. In children under 3 years of age more than 50 percent of cases have been reported to occur due to Pseudomonas unlike adults where 19 percent of the cases occurred due to the same.

Apart from these, there are myriad of other organisms, which may be isolated from cases of microbial keratitis in children (Table 11.3).

Childhood microbial keratitis may also be caused by polymicrobial infections, which varies from 6.9 to 27 percent (Table 11.4).

Viruses

Viral keratitis may also occur in children. Herpes simplex virus infection is the most common. Primary herpetic infections as well as recurrences of the same may occur. Herpes simplex infection has been associated in over one third of all cases of corneal ulceration in children in one study. Primary herpes manifests as typical vesiculated herpetic skin lesions, preauricular lymphadenopathy and follicular conjunctivitis with or without corneal involvement.

Those with recurrences have a previous episode of typical primary herpes and are characterized by herpetic epithelial involvement alone, stromal keratitis or both. Epithelial involvement varies from punctuate epithelial keratitis, areolar-stellate, linear dendritic or ameboid.

Fungus

In children the incidence of fungal keratitis is less as compared to bacterial keratitis. Fungal infections may occur in 17.5 to 18.2 percent of the cases (Table 11.3). Aspergillus and Fusarium species are the most frequently identified organisms (Table 11.3).

NON-MICROBIAL KERATITIS

Nutritional

Malnourished children are more prone to vitamin A deficiency and measles induced corneal ulceration. Vitamin A deficiency leading to keratomalacia is the most important cause of bilateral corneal ulceration. The ulcers vary from small punched out, round, or oval ulcers involving corneal stroma to complete necrosis of the cornea.

Vernal Keratoconjunctivitis

The abnormalities of ocular immune mechanisms found in vernal keratoconjunctivitis predispose these patients to microbial keratitis. Vernal keratoconjunctivitis may be rarely associated with secondary bacterial infection and fungal keratitis.
### Specific Types of Keratitis

#### TABLE 11.3
Non-viral microbes identified from cases of microbial keratitis in children

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ormerod⁴</th>
<th>Cruz¹⁰</th>
<th>Clinch⁵</th>
<th>Kunimoto¹</th>
<th>Vajpayee⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Gram-positive cocci</td>
<td>89.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>34.1</td>
<td>54</td>
<td>75.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17</td>
<td>2.3</td>
<td>5</td>
<td>23.4</td>
<td>60</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>17</td>
<td>20.5</td>
<td>1</td>
<td>20.3</td>
<td>8</td>
</tr>
<tr>
<td><em>Streptococci</em> species</td>
<td>20</td>
<td>6.8</td>
<td>2</td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Total Gram positive bacilli</td>
<td>2.3</td>
<td></td>
<td></td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td><em>Corynebacterium</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.3</td>
</tr>
<tr>
<td><em>Bacillus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>Total gram-negative cocci</td>
<td>0</td>
<td>6.8</td>
<td>21</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Total gram-negative bacilli</td>
<td>43.2</td>
<td></td>
<td></td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>24</td>
<td>34.1</td>
<td>2</td>
<td>9.4</td>
<td>14.5</td>
</tr>
<tr>
<td><em>Pseudomonas</em> species</td>
<td></td>
<td></td>
<td></td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter</em> species</td>
<td>2</td>
<td>4.5</td>
<td>1</td>
<td>3.1</td>
<td>3</td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td><em>Aeromonas</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td><em>Hemophilus</em> species</td>
<td>2</td>
<td>2.3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> species <em>Klebsiella</em> species</td>
<td></td>
<td>4.5</td>
<td>1</td>
<td>3.1</td>
<td>3</td>
</tr>
<tr>
<td><em>Branhamella</em> species</td>
<td></td>
<td>4.5</td>
<td>1</td>
<td>3.1</td>
<td>3</td>
</tr>
<tr>
<td>Total Gram-negative bacilli-Non-fermenting</td>
<td>7</td>
<td></td>
<td></td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td><em>Moraxella</em> species</td>
<td></td>
<td>2.3</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Moraxella lacunata</em></td>
<td></td>
<td></td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total parasites</td>
<td>8</td>
<td></td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acanthamoeba</em> species</td>
<td>2</td>
<td></td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fungus</td>
<td>18.2</td>
<td></td>
<td>17</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>Total filamentous fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.1</td>
</tr>
<tr>
<td><em>Aspergillus</em> species</td>
<td>2</td>
<td>4.5</td>
<td>1</td>
<td>6.3</td>
<td>8.6</td>
</tr>
<tr>
<td><em>Fusarium</em> species</td>
<td>2</td>
<td>11.4</td>
<td>1</td>
<td>3.1</td>
<td>5.7</td>
</tr>
<tr>
<td><em>Unidentified</em> species</td>
<td></td>
<td></td>
<td>2.3</td>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td><em>Curvularia</em> species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total yeast like fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> species</td>
<td>1</td>
<td></td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

### Diagnosis

The child with suspected microbial keratitis should preferably be immediately hospitalized. A detailed history should be taken and a meticulous clinical examination should not only include a detailed ocular examination but also a relevant systemic evaluation.

#### HISTORY

The history may be elicited from the patient if the child is older or the parent if the child is younger. History should be taken regarding the onset, duration and the possible predisposing factors of corneal ulceration. History of prior treatment with topical medications (including antimicrobial and corticosteroids) should also be sought.

Apart from this, history should also be obtained regarding any fever, rash or diarrhea prior to the onset of or during the occurrence of corneal ulceration. The immunization status of the child should also be found out especially against measles.
In cases of contact lens wear, specific history should be taken regarding the indication type and duration (daily wear or extended wear) of contact lens wear and the contact lens hygiene including the cleaning and disinfection regime.

**CLINICAL EXAMINATION**

Clinical examination of the child should not only include a detailed ocular examination but also relevant systemic examination.

**Systemic Examination**

The systemic examination of the child should be done with special reference to the nutritional status of the child. Features suggestive of malnutrition or Vitamin A deficiency should be evaluated and the child may also be examined by a pediatrician in such cases.

In suspected herpetic keratitis systemic features such as typical vesiculated skin lesions and preauricular lymphadenopathy should also be evaluated.\(^{11}\)

**Ocular Examination**

Standard protocol should be followed and daily slit lamp biomicroscopic examination should be undertaken. Children are difficult to examine with the slit lamp, as they are uncomfortable, fearful and photophobic. Attempts to ameliorate their anxiety with toys and games should be made. In case they do not allow a routine examination, alternative examination under sedation and examination under anesthesia may also be considered, the details of which are described later.

**Slit-lamp Biomicroscopy**

A slit-lamp biomicroscopy is performed using a routine slit lamp microscope or a portable slit lamp microscope (Fig. 11.3). There are several useful aids for the routine slit lamp examination, which have been recommended.\(^{12}\) The base plate for Hruby lens of the Haag Streit slit lamp should be removed because it tends to press into the protuberant abdomen of the thrusting or the struggling child. Technicians or nurses should not be allowed to hold the infant. The mother of the child should be encouraged to hold the child in her lap. The head of the child should be steadied but extremely forceful pressing is discouraged since respiratory distress may occur due to the compression of the laryngeal structures against the chin rest.

The size, extent and depth of the corneal ulcer, including the epithelial defect as well as the infiltration should be noted and documented (Figs 11.1 and 11.2). Fluorescein or Rose Bengal may directly be administered rather than by controlled small drops from a glass rod or applicator stick to avoid injury. An associated hypopyon or uveitis and involvement of the sclera, if any should also be documented.

Ulcer characteristics of microbial keratitis in various studies is shown in Table 11.4.

**INVESTIGATIONS**

Investigation in a case of pediatric keratitis include the usual laboratory investigations for microbiological diagnosis and relevant systemic investigations in selected cases.

![Figure 11.1: Bacterial keratitis in a child](image1)

![Figure 11.2: Perforated corneal ulcer in a child](image2)
Specific Types of Keratitis

**TABLE 11.4**

<table>
<thead>
<tr>
<th>Ulcer characteristics (%)</th>
<th>Ormerod⁴</th>
<th>Cruz¹⁰</th>
<th>Clinch⁵</th>
<th>Kunimoto¹</th>
<th>Vajpayee⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hypopyon</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>2. Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>47</td>
<td>63</td>
<td>52</td>
<td>52</td>
<td>58</td>
</tr>
<tr>
<td>Paracentral</td>
<td>49</td>
<td>33</td>
<td>31</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Peripheral</td>
<td>4</td>
<td>4</td>
<td>14</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>3. Size of ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 mm</td>
<td>30</td>
<td>25</td>
<td>31</td>
<td>31</td>
<td>51</td>
</tr>
<tr>
<td>2-6 mm</td>
<td>49</td>
<td>61</td>
<td>52</td>
<td>51</td>
<td>16</td>
</tr>
<tr>
<td>&gt;6 mm</td>
<td>21</td>
<td>14</td>
<td>14</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

**Systemic Investigations**

Nutritional status has been evaluated by comparing weight for age against Harvard standards in western literature¹¹ and deficit in weight for height in Indian literature.⁷

Serum Vitamin A levels may be measured by using high-performance liquid chromatography, although this investigation is not mandatory routinely.¹¹

For herpes simplex keratitis enzyme-linked immuno-sorbent assay cultures may be obtained, although these are not mandatory and should be only undertaken in case of a diagnostic dilemma.¹²

**Laboratory Investigations**

Many ophthalmologists assume that it is difficult to obtain a corneal specimen of a child and therefore do not adhere to the standard protocol for obtaining a specimen before starting antibiotic therapy. In children lack of patient co-operation should not preclude corneal scraping. Corneal scraping is feasible provided it is done under appropriate anesthesia.

Corneal scrapings may be obtained either under topical anesthesia, topical anesthesia and sedation or general anesthesia depending on the age of the child and his/her co-operation.

**Topical Anesthesia**

For topical anesthesia either 4 percent xylocaine or 0.5 percent proparacaine is used. The latter is preferred as it is least epitheliotoxic.

**Topical Anesthesia and Sedation**

In most cases of pediatric keratitis, an additional sedation is required. Choral hydrate (50-100 mg/kg body weight) is very commonly used for sedation.⁵ Sedation may also be achieved by a single intramuscular injection of meperidine (2 mg/kg), promethazine (2 mg/kg) and chlorpromazine (1 mg/kg). Injection diazepam 0.1 mg/kg has also been used for sedation.¹⁰

**General Anesthesia**

In unco-operative children general anesthesia should be used to obtain scraping.

Corneal scrapings should be obtained either with the platinum spatula,³ Bard Parker blade¹ or with the bent 26-gauze needle⁹ from the base as well as the leading edge of the ulcer. Routine Gram’s staining and KOH
wet mount preparation is done to examine the smears of corneal scrapings. The specimens obtained should be sent for inoculation onto sheep blood agar, chocolate agar and thioglycolate broth, all incubated at 37 deg C. Sabouraud’s agar plates supplemented with yeast extract and 50 micrograms/ml gentamicin sulphate (without cyclohexamide) should also be inoculated at 20°C to check for fungal infection. In suspected cases for Acanthamoeba, non-nutrient agar overlaid with Escherichia Coli should be inoculated.

The culture positivity and polymicrobial involvement in pediatric microbial keratitis is shown in Table 11.5.

Management

The management of a case of pediatric keratitis should include not only the standard medical therapy for ocular affection but also for systemic diseases.

Attention should be particularly given to the nutrition and vitamin A therapy especially in cases of keratomalacia. All children with keratomalacia should receive vitamin A supplement as per the WHO recommendation.

Vitamin A supplementation can be given orally, parenterally or both. The initial dose is 200,000IU in oil (110 mg of retinal palmitate or 66mg retinal acetate) or, if necessary, 200,000 IU of water soluble vitamin A intramuscularly. The next day an additional 200,000 IU of vitamin A should be given. For children less than one year of age half of these doses are given. Children who are at high risk may require a repeat dose of vitamin A at 4 to 6 months.

### Table 11.5

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Author (Year)</th>
<th>Culture positivity (%)</th>
<th>Polymicrobial involvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ormerod et al (1986)</td>
<td>87</td>
<td>27</td>
</tr>
<tr>
<td>2.</td>
<td>Cruz et al (1993)</td>
<td>86.3</td>
<td>11.7</td>
</tr>
<tr>
<td>3.</td>
<td>Clinch et al (1994)</td>
<td>75.8</td>
<td>6.9</td>
</tr>
</tbody>
</table>

### Table 11.6

<table>
<thead>
<tr>
<th>Therapy in cases of microbial keratitis in children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ormerod</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Medical therapy</td>
</tr>
<tr>
<td>Surgical intervention</td>
</tr>
</tbody>
</table>

### Table 11.7

<table>
<thead>
<tr>
<th>Safe Anti-microbials in pediatric age group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibacterial agents</strong></td>
</tr>
<tr>
<td>1. Ciprofloxacin eye drops 3 mg/ml</td>
</tr>
<tr>
<td>2. Ofloxacin eye drops 3 mg/ml</td>
</tr>
<tr>
<td>3. Tobramycin 14 mg/ml</td>
</tr>
<tr>
<td>4. Gentamicin 14 mg/ml</td>
</tr>
<tr>
<td>5. Amikacin 20 mg/ml</td>
</tr>
<tr>
<td>6. Cefazolin 50 mg/ml</td>
</tr>
<tr>
<td>7. Ceftazidime 50 mg/ml</td>
</tr>
<tr>
<td>8. Chloramphenicol 3 mg/ml</td>
</tr>
<tr>
<td>9. Vancomycin 50 mg/ml</td>
</tr>
<tr>
<td><strong>Antifungal agents</strong></td>
</tr>
<tr>
<td>10. Natamycin 50 mg/ml</td>
</tr>
<tr>
<td>11. Amphotericin B 0.1-0.25%</td>
</tr>
<tr>
<td><strong>Antiviral agents</strong></td>
</tr>
<tr>
<td>12. Vidarabine 3 mg/ml</td>
</tr>
<tr>
<td>13. Acyclovir 3 mg/ml</td>
</tr>
</tbody>
</table>

### MEDICAL THERAPY

Most ulcers respond to medical therapy alone, although penetrating keratoplasty may be required in some eyes (Tables 11.6 and 11.7). Concern for maintenance of adequate corneal antibiotic levels by topical application in crying, unco-operative children has led some researchers to advocate repeated sub-conjunctival injections under chloral hydrate sedation. However, we do not generally recommend the use of sub-conjunctival antibiotics to treat microbial keratitis.

### Bacterial Keratitis

We advocate that initial medical therapy should include a combination of fortified antibiotic drops of cefazolin sodium (5%) and tobramycin sulphate (1.3%) at frequent intervals which may vary initially from half hourly to two hourly intervals for the initial 48 hours. Subsequent
Specific Types of Keratitis

modifications in the choice of antibiotics and their dosage depend on the results of culture and sensitivity pattern and clinical response. Alternatively, monotherapy with ciprofloxacin eye drops 0.3 percent may also be used if the ulcers are small and do not involve pupillary axis.

Fungal Keratitis

In case of identification of fungus in a smear, 5 percent natamycin drops should be instilled at 5 times a day along with the antibacterial medications.

Tablet ketoconazole (200 mg) may be administered twice daily (after liver function tests) in patients in whom the fungal corneal ulcer is greater than 6 mm in diameter, deeper than anterior half of stroma or if anterior chamber exudates are present.

Herpetic Keratitis

For herpetic keratitis in children, topical application of acyclovir ointment five times a day is given. However, in crying and unco-operative children this may be a problem and so debridement alone as an alternative may be tried (especially in clinics where outpatient anesthesia is available). Debridement should be done under the microscope, and a cycloplegic agent and the eye is patched. Oral Acyclovir in children < 8 years of age should be used as an adjunct in addition to topical therapy in the dose of 12-20 mg/kg body weight. It is effective in treating active infectious epithelial keratitis, in prophylaxis of children prone to recurrent infectious epithelial disease and for prevention of infectious disease in children being treated with topical corticosteroids medications for immune stromal keratitis.

Topical steroids are rarely necessary, but parents should be alerted to the symptoms of recurrent stromal keratitis such as photophobia. Recurrences should be treated promptly with assessment of the severity of the stromal keratitis. Corticosteroids should be employed when the lesions are central and severe and selectively in cases of marginal stromal keratitis as this may induce irregular astigmatism without their use. With the use of corticosteroids, attention should be given to the possible side effects, which should include ocular hypertension and possible subcapsular cataracts. Their use requires slit lamp examination, frequent observation of the optic discs through dilated pupils and evaluation of intraocular pressure on a regular basis.

Acanthamoeba Keratitis

Cases of Acanthamoeba keratitis are treated with topical polyhexamethylene biguanide (0.02%) drops and 0.1% propamidine isethionate every hour for 3 days. The therapy is subsequently tapered. Improvement is seen within 2-3 weeks following which qid doses of drugs are given.

Apart from the antimicrobial agents, supportive therapy should include cycloplegics, antiglaucoma drugs and lubricants.

FOLLOW-UP

Follow-up and monitoring of these patients should be done daily and sedatives and general anesthesia should be used whenever and wherever it is deemed necessary for such examinations.

Absence of symptoms, disappearance of circumcorneal congestion, absence of infiltration and lack of fluorescein staining are criteria for healed corneal ulcer.

SURGICAL THERAPY

Surgery for the management of pediatric corneal ulceration may also be required in some cases. Surgical intervention may be required in 4 to 28 percent of the eyes as highlighted in various studies (Table 11.6). In a study from our center the pediatric keratoplasty was done for infectious keratitis and keratomalacia in nearly two-thirds of the eyes.

Apart from penetrating keratoplasty, lamellar keratoplasty, cyanoacrylate glue application, conjunctival flap and enucleation may be undertaken in these cases, as deemed necessary. The visual prognosis after penetrating keratoplasty in children for microbial keratitis is generally poor. Clear grafts have been reported in only 12.5 percent of the cases requiring keratoplasty in one study.

POST-RESOLUTION MANAGEMENT

The various outcomes following microbial keratitis in children include corneal scars, adherent leukomas, glaucoma, anterior staphyloma, and disorganized globe. However, the most frequent outcome is the presence of a corneal scar. The visual acuity after resolution/treatment of pediatric keratitis depends on the severity of the corneal ulcer and the promptness of therapy. Poorer visual
acuities may be obtained, which does not commensurate with the extent of the corneal involvement. This has been attributed to the anisometropic or stimulus deprivation amblyopia and permanent failure to develop stereopsis especially if the corneal opacification occurs in the early years. Hence, the ophthalmologist should be vigilant in the postoperative period to institute anti-amblyopia therapy as and when required.

PREVENTION

In general accidents and injuries in children should be avoided and precautions should be undertaken to prevent the same. Likewise Vitamin A deficiency should be prevented. Sufficient Vitamin A should be available in the diet and supplementation should be done whenever required as per the WHO guidelines. Early diagnosis and treatment of Vitamin A should be a priority in areas where protein energy malnutrition is prevalent. Complete immunization protocol for children should be followed and children should be especially immunized against measles. Use of traditional eye medications for minor eye ailments should be discouraged. Any eyes with prior ocular surface disease or anterior segment surgery should be under vigilance and an early diagnosis and prompt treatment of corneal ulcers in these cases is mandatory. In cases of contact lens wear the parents and the children should be educated about the contact lens hygiene and proper handling. The pediatrician and the neonatologist should be made aware of the predisposing factors of keratitis.

References

Contact Lens Related Keratitis

Introduction
Contact lens induced keratitis is an ocular emergency that requires immediate and appropriate treatment to limit corneal morbidity and vision loss. Contact lens wear is the most common predisposing factor for infectious keratitis in patients with previously healthy eyes.

Epidemiology
The incidence rates for bacterial microbial keratitis range from approximately 2/10,000 per year for rigid contact lens, 2.2-4.1/10,000 per year for daily-wear soft contact lens, to 13.3-20.9/10,000 per year for extended-wear soft contact lenses. The risk with therapeutic contact lenses is much higher: 52/10,000 per year.

Risk Factors
The major risk factors in the development of contact lens related infectious keratitis include corneal trauma, exposure to polluted water, use of home made saline for disinfection, improper lens care and poor compliance with contact lens cleaning solutions, overnight wear and extended wear schedules.

Overnight usage for soft contact lenses is associated with a higher risk and this increases with increasing number of nights of continuous wear.

Higher risks of contact lens induced keratitis have also been related to lower socioeconomic status, smoking and patients with the acquired immunodeficiency virus.

Pathogenesis
The pathogenesis of the contact lens induced keratitis is related to the factors related to the alterations produced in the dynamics of the tear film and the ocular surface due to contact lenses, contact lenses per se and the contact lens care systems.

Altered Tear Dynamics Due to Contact Lenses
Contact lenses have significant effect on the tear film, resulting in increased tear evaporation, stimulation of reflex tearing, a decreased blink rate, alteration in tear-film osmolarity and trapped debris with tear stagnation under the lens. Epithelial erosions from corneal desiccation manifest as coarse punctate erosion in the central or paracentral cornea or with nasal and temporal peripheral staining of the cornea adjacent to the edge of the lens.

The normal cornea obtains oxygen by diffusion from air when the eye is open and from the tarsal and bulbar conjunctiva when the eye is closed. Contact lens wear causes a wide spectrum of changes in the cornea and conjunctiva such as an induced hypoxia and hypercapnia. This causes a reduction in the corneal epithelial metabolic rate and accumulation of stromal lactic acid which leads to a compromised epithelial junctional integrity, resulting in epithelial fragility, abrasion, punctate keratitis and susceptibility to microbial invasion. A more permanent effect of stromal acidosis is endothelial polymegathism, characterized by an increased variation in corneal endothelial cell size. More severe changes of polymegathism occur with increased duration of contact lens wear.

Additionally, allergic or toxic responses can occur related to deposits that form on the lens surface or to reactions against sensitizing preservative solutions.

Contact Lenses
The presence of contact lenses and the contact lens deposits increases bacterial adherence. Multiple factors play a role in mediating bacterial adherence to the
contact lens including net surface charge of bacteria, carbohydrate adhesions, the biofilm, exotoxins and endotoxins. Bacteria have mechanisms to adhere to contact lens surfaces, especially worn lenses with components of mucin and proteins from the tear film.10 The rapid production of a biofilm on the lens further increases the bacterial attachment. The adherence of bacteria on the lens surface allows the development of a glycocalyx and biofilm to form and convert adhering bacteria into replicating bacteria colonizing the lens surface with a stronger attachment. The glycocalyx is a polysaccharide (slime) containing structure lying outside the outer membranes of Gram-positive and Gram-negative organisms.11,12 Organisms can also reach the contact lens from the conjunctival flora or other environmental sources.13 These biofilms may explain how complaint patients or patients with uncontaminated contact lens cases can have a source of organisms.

The insertion and removal process can injure the cornea by lens deposits or defects, by chemical toxicity of contact lens disinfectants, or by lens-induced hypoxia. Oxygen transmissibility is also an important factor in contact lens associated ulcerative keratitis. The chances of visually significant infections with overnight wearing of contact lenses are always significant.

CONTAMINATION OF CONTACT LENS CARE SYSTEMS

Contact lens case contamination results from a combination of poor hygiene and the failure of current disinfection systems.14-18 Disinfection by chlorine is associated with a higher contamination rate compared with chemical, hydrogen peroxide, chlorhexidine, or heat.19 Contamination of rigid gas permeable lens cases is associated with increasing age of the contact lens. The use of contaminated tap water or home made saline solutions may also cause keratitis.17,20 Other features which influence the contamination of the lens case include the patient’s hygiene and the surrounding socioeconomic environment.21

Biofilm formation has been demonstrated on contact lenses and contact lens cases even after appropriate sterilization.13, 20

Organisms

Although the bacterial infections are most prevalent, fungi, parasites and viruses can also cause the infections.5 Approximately one third of contact lens related microbial keratitis is associated with Gram-positive cocci, such as Staphylococci and Streptococci, but two thirds is associated with gram-negative rods, especially Pseudomonas aeruginosa.5-7,14,15 Fungi are relatively infrequent and account for 3 percent of infections, occurring more commonly following the usage of therapeutic contact lenses as compared to the cosmetic or aphakic contact lenses. Acanthamoeba keratitis has been associated with both hard and soft contact lenses, although daily wear soft contact lens wearers are more frequently involved.14-18

CLINICAL FEATURES

Patients usually present with symptoms of progressive discomfort, limbal or conjunctival hyperemia, photophobia, muco-purulent exudates and decreased vision. The contact lens induced keratitis may manifest as an infiltrative keratitis or an ulcerative keratitis. The infiltrates associated with contact lens wear may or may not be clinically significant Sweeney et al have classified the events related to contact lens wear into serious events, clinically significant events and clinically non significant events22 (Table 12.1).

Microbial Keratitis

The symptoms in cases of microbial keratitis include severe limbal and bulbar redness, rapid onset of moderate to severe pain, decreased visual acuity, muco-purulent or purulent discharge, tearing, photophobia, and lid edema.

Microbial keratitis in a contact lens wearer is characterized by excavation of the corneal epithelium, Bowman’s layer, and stroma with infiltration and necrosis of tissue. Focal infiltrates are usually large (> 1 mm) and irregular with small satellite lesions and significant diffuse infiltration. Infiltrates are mainly found in the central or paracentral region.
Specific Types of Keratitis

Contact Lens Induced Peripheral Ulcer
The symptoms of a case of contact lens induced peripheral ulcer (CLPU) include limbal and bulbar redness and tearing. Patients experience severe to moderate pain, foreign body sensation, or irritation or could even be asymptomatic.

It is associated with an inflammatory reaction of the cornea characterized in its active stage by focal excavation of the epithelium, infiltration, and necrosis of the anterior stroma. Small (up to 2 mm), single, circular focal infiltrates with slight diffuse infiltration surrounding the focal infiltrates are present in the midperiphery to periphery of the cornea (Figs 12.1 and 12.2).

Contact Lens Induced Red Eye
The symptoms in a case of contact lens induced red eye include moderate to severe circumferential redness, irritation to moderate pain, tearing, and photophobia. Patients are awakened by their symptoms or notice them soon after waking.

There is an inflammatory reaction of the cornea and the conjunctiva immediately following eye closure. Small multiple focal infiltrates and diffuse infiltration in the midperiphery to periphery of the cornea are present, generally without punctate staining overlying the infiltrate.

Infiltrative Keratitis
The symptoms in a case of infiltrative keratitis include mild to moderate irritation, redness, and occasional discharge.

Infiltrative keratitis there is an inflammatory reaction of the cornea characterized by anterior stromal infiltration, with or without epithelial involvement, in the mid periphery to periphery of the cornea. Infiltrates are small, possibly multiple, with or without accompanying mild to moderate diffuse infiltration.

Asymptomatic Infiltrative Keratitis
Asymptomatic infiltrative keratitis is defined as an inflammatory event characterized by infiltration of the cornea without patient symptoms.

There are small focal infiltrates (up to 0.4 mm) with or without mild to moderate diffuse infiltration in the periphery of the cornea. It is associated with punctate staining after instillation of fluorescein and may be associated with mild to moderate limbal and bulbar redness.

Asymptomatic Infiltrates
There are very small focal infiltrates (0.2 mm) and/or mild, diffuse infiltration with no staining overlying the infiltrates after instillation of fluorescein. Infiltrates were commonly peripheral but could be found across the cornea (Fig. 12.3).

Management
The management of contact lens induced keratitis consists of management of microbial keratitis and infiltrates.
Corneal scrapings for smears and culture should be obtained prior to initiation of antimicrobial therapy for infectious keratitis. Specimens should be directly inoculated onto standard media – blood, chocolate, and Sabouraud dextrose agar and thioglycolate broth. KOH and Gram-stained smears of corneal scrapings help in the identification of pathogens such as Staphylococcus (Fig. 12.4) Pseudomonas (Fig. 12.5) and Acanthamoeba (Fig. 12.6) as well as fungal elements. In contact lens wearers, culturing lens care solutions and the contact lens care may be helpful in establishing the diagnosis.

Although Acanthamoeba cysts and trophozoites obtained from corneal scrapings can be stained with Gram stain, as well as with giemsa, trichrome, periodic acid – Schiff (PAS), Gomori methenamine silver (GMS), and hematoxylin and eosin stain, calcofluor white a chemofluorescent vital stain, is also very useful. It binds to chitin and cellulose in the cell walls of fungi and Acanthamoeba cysts. Yeast, fungal elements and Acanthamoeba cysts show bright apple-green fluorescence against a reddish-orange background. An indirect immunofluorescent staining technique and fluorescien-conjugated lectins may also be used for Acanthamoeba species identification, but these
techniques require a fluorescent microscope for visualization.

Corneal scrapings inoculated on a non-nutrient agar with an overlay of *Escherichia coli* or other Gram-negative organism enhances the recovery of Acanthamoeba. The presence of Acanthamoeba trophozoites is indicated by a snail-tract clearing through the layer of bacteria.

Antibiotic therapy is instituted according to the isolated organism. Fortified cefazolin sodium 5 percent and tobramycin 1.3 percent is initiated after preliminary corneal scraping is done. Topical antibiotic therapy is altered in accordance to the culture sensitivity reports and clinical progress. They are given at one hourly frequency for the initial 48 hours. Following a clinical response the fortified antibiotics are tapered to 2 hourly pregnancy and subsequently 4 hourly. Anti-acanthamoeba treatment is initiated in cases with positive Acanthamoeba cysts. The long-term maintenance of anti-acanthamoeba therapy is important along with close and regular follow-up.

**STERILE INFILTRATES**

If a sterile infiltrate is suspected, the contact lens should be removed immediately. A topical antibiotic such as a fluoroquidone (0.3% ofloxacin) or 0.3% ciprofloxacin qic should be used which is effective against *Pseudomonas* and the eye should be re-examined after 24 hours. In our experience, discomfort is substantially reduced within 24 hours of removing the contact lens, if the infiltrate is sterile.

Withholding antibiotic therapy for 24 hours after the contact lens is removed is an alternative approach. This approach, without the addition of other therapy should be undertaken only when the ophthalmologist is very sure that the patient will return 12-24 hours after the initial examination. If, by this time, the patient discomfort increases as does fluorescein staining, stromal edema, and infiltration, therapy for bacterial infection is initiated.

However, if symptoms diminish or abate after the initial 12-24 hours and if the corneal signs decrease, therapy is again withheld and the patient is asked to return again in 24 hours for further observation. After 48 hours of observation without therapy, a sterile infiltrate typically becomes more translucent and, over the next several days, will continue to improve or resolve. In such instances, when the patient is symptom-free (especially if the infiltrate is outside the visual axis), there is no need for further treatment. However, if discomfort or disturbed vision continues to persist, the patient may demand a more rapid resolution of symptoms. In these rare situations, topical corticosteroid therapy may be administered. We suggest commencing topical corticosteroid treatment, usually two to three drops of 1 percent prednisolone acetate daily, only after ocular symptoms have diminished spontaneously after removal of the contact lens and there is no increase in fluorescein staining, stromal edema, or infiltrate size or density over the course of 2-3 days after the initial diagnosis. It is mandatory to attempt this approach only in those patients who can be confided to comply with follow-up schedules and who can be seen daily for the first few days.

**PREVENTION**

Disinfection resistant biofilms must be disrupted regularly by scrubbing all internal surfaces of the contact lens case with a cotton ball or Q-tip moistened with the contact lens cleaner. The contact lens case should be heat disinfected with hot water exposure periodically, followed by air-drying and the case should be replaced periodically. We recommend the use of daily disposable contact lenses as they are associated with decreased chances of infection.

**References**

8. Carlson KH, Bourne WM, Brubarker RF. Effect of long term contact lens wear on corneal endothelial cell
Contact Lens Related Keratitis

Infectious crystalline keratopathy is a specific entity seen most frequently following keratoplasty. It manifests as an indolent corneal infection with needle-like branching crystalline opacities. Although there are no actual deposition of crystals, the term “crystalline” refers to the characteristic appearance of these lesions.

Risk Factors

The most common risk factor responsible for the occurrence of ICK is prior ocular surgery and steroid use. ICK never occurs as a primary disease in a previously healthy cornea. It occurs most often following penetrating keratoplasty although it has also been reported after other surgeries such as epikeratoplasty, corneal relaxing incisions, LASIK and following glaucoma filtering surgery with postoperative subconjunctival 5-fluorouracil (Table 13.1).

ICK may also occur in eyes with post-herpetic persistent epithelial defects treated with bandage soft contact lenses and topical anesthetic abuse. One or more of these factors may also co-exist. In cases of penetrating keratoplasty, acute herpes simplex keratitis, Acanthamoeba keratitis and topical anesthetic abuse, persistent epithelial damage occurs, which acts as a portal of entry for bacteria.

Systemic and topical immunosuppression have also been known to be predisposing factors for the development of ICK. Eyes, which have been previously treated with long-term topical corticosteroids, and topical antimicrobials are also predisposed.

Etiology

ICK is usually caused by an alpha-hemolytic streptococcus in majority of cases (Table 13.2). Other causative bacteria which have been reported include peptostreptococcus, nutritionally variant Streptococci, haemophilus aphrophilus, coagulase-negative staphylococci, pseudomonasi propionibacterium acnes and mixed infection with Mycobacterium fortuitum and pseudomonas aeruginosa and streptococcus pneumoniae (Table 13.3).

Fungal organisms may also cause ICK. These include Candida albicans (mixed with Staphylococcus epidermidis

Introduction

Infectious crystalline keratopathy is a specific entity seen most frequently following keratoplasty. It manifests as an indolent corneal infection with needle-like branching crystalline opacities. Although there are no actual deposition of crystals, the term “crystalline” refers to the characteristic appearance of these lesions.

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### Table 13.1 Factors associated with ICK

<table>
<thead>
<tr>
<th>Ocular factors</th>
<th>Ocular surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Corneal transplant (most common)</td>
<td></td>
</tr>
<tr>
<td>2. Epikeratophakia</td>
<td></td>
</tr>
<tr>
<td>3. Relaxing incisions</td>
<td></td>
</tr>
<tr>
<td>4. Glaucoma filtering surgery with postoperative subconjunctival 5-fluorouracil</td>
<td></td>
</tr>
<tr>
<td>5. Laser in situ keratomileusis</td>
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<table>
<thead>
<tr>
<th>Ocular surface disorders</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1. Persistent epithelial defect</td>
<td></td>
</tr>
<tr>
<td>2. Chronic herpetic keratitis</td>
<td></td>
</tr>
<tr>
<td>3. Ocular cicatricial pemphigoid</td>
<td></td>
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<tr>
<td>4. Corneal scars</td>
<td></td>
</tr>
<tr>
<td>5. Acanthamoeba keratitis</td>
<td></td>
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<tr>
<td>6. Exposure keratitis</td>
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<table>
<thead>
<tr>
<th>Topical drugs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Corticosteroids (Prednisolone acetate, betamethasone, dexamethasone)</td>
<td></td>
</tr>
<tr>
<td>2. Antibiotics (Bacitracin eye ointment, neo-poly B., Sulphacetamide, gramacidine, gentamicin, tobramycin, ciprofloxacin, cefazolin)</td>
<td></td>
</tr>
<tr>
<td>3. Anesthetic abuse-Proparacaine HCl</td>
<td></td>
</tr>
<tr>
<td>4. Antiglaucoma-timolol maleate, apraclonidine</td>
<td></td>
</tr>
<tr>
<td>5. Antivirals-Trifluridine</td>
<td></td>
</tr>
<tr>
<td>6. Others-atropine, cyclosporin, NaCl ointment, unpreserved tears, chlorhexidine.</td>
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<table>
<thead>
<tr>
<th>Systemic factor</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1. Systemic immunosuppression</td>
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</table>

aphrophilus, coagulase-negative staphylococci, pseudomonasi propionibacterium acnes and mixed infection with Mycobacterium fortuitum and pseudomonas aeruginosa and streptococcus pneumoniae (Table 13.3).

Fungal organisms may also cause ICK. These include Candida albicans (mixed with Staphylococcus epidermidis

aphrophilus, coagulase-negative staphylococci, pseudomonasi propionibacterium acnes and mixed infection with Mycobacterium fortuitum and pseudomonas aeruginosa and streptococcus pneumoniae (Table 13.3).

Fungal organisms may also cause ICK. These include Candida albicans (mixed with Staphylococcus epidermidis
Infectious Crystalline Keratopathy

**Pathogenesis**

Predilection for alpha-hemolytic streptococci to cause infection in ICK is unknown. These bacteria are the predominant inhabitants of the oral cavity and upper airway and may rarely inhabit the normal flora of the eyelids or conjunctiva. Access to the corneal stroma appears to occur via breaks in the corneal surface or through sutures.

It is unclear whether this is due to intrinsic properties of the organisms or whether the corneal stroma provides a marginal environment for bacterial replication. Glycocalyx production which is characteristically seen in these cases may shield the organisms from immune recognition, decreasing the inflammatory response, which is suppressed further by the chronic use of topical corticosteroids.

Although the reason for the crystalline growth pattern of the lesions is not known, it is possibly related to the criss-cross lamellar architecture of the corneal stroma and represents the path of least resistance to the expanding bacterial colonies.

Characteristically intralamellar pockets of bacteria within the corneal stroma are isolated in a single lamellar plane. There is a distinct paucity of inflammatory cells of any type. The overlying epithelium is often intact, if an accompanying disease process has not altered it. Collagen lamellae adjacent to the collections of bacteria are undisturbed with no evidence of necrosis or corneal thinning. The crystalline deposits, which are composed of bacterial aggregates, are most often in the anterior and less commonly in a mid-stromal location or in posterior stroma. Intraocular spread of organisms may occur rarely.

**Clinical Features**

ICK has been reported worldwide, with no predilection for any race. Primarily, it is a disease of the adults and is rare in patients under 20 years of age. Usually it presents unilaterally, although anecdotal reports of bilateral presentation are also there.

ICK occurs most often following a keratoplasty and the lesions often originate at the site of a suture track.

<table>
<thead>
<tr>
<th>TABLE 13.2</th>
<th>Microbial organisms isolated from eyes with ICK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Bacteria</strong></td>
<td>1. Gram-positive aerobic.</td>
</tr>
<tr>
<td></td>
<td>Streptococcus (42%)</td>
</tr>
<tr>
<td></td>
<td>Streptococcus viridans and variants (mitis, sanguis)</td>
</tr>
<tr>
<td></td>
<td>Enterococcus (Streptococcus fecalis)</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus spp. (12%) (aureus, hemolyticus, Epidermidis)</td>
</tr>
<tr>
<td></td>
<td>Micrococcii</td>
</tr>
<tr>
<td></td>
<td>2. Gram-positive aerobic bacilli</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium fortuitum</td>
</tr>
<tr>
<td></td>
<td>Bacillus spp</td>
</tr>
<tr>
<td></td>
<td>3. Gram-positive anaerobic cocci</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus</td>
</tr>
<tr>
<td></td>
<td>4. Gram-positive anaerobic bacilli</td>
</tr>
<tr>
<td></td>
<td>Diphtheroids</td>
</tr>
<tr>
<td></td>
<td>Corynbacterium Spp</td>
</tr>
<tr>
<td></td>
<td>Propionibacterium acne</td>
</tr>
<tr>
<td></td>
<td>5. Gram-negative aerobic rods</td>
</tr>
<tr>
<td></td>
<td>Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td></td>
<td>6. Gram-negative bacilli</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas maltophilia</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas stutzeri</td>
</tr>
<tr>
<td><strong>II. Fungus (8%)</strong></td>
<td>1. Yeast like Fungi</td>
</tr>
<tr>
<td></td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td></td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td></td>
<td>2. Filamentous fungi (dematiaceous)</td>
</tr>
<tr>
<td></td>
<td>Alternaria</td>
</tr>
<tr>
<td><strong>III. Acanthamoeba species</strong></td>
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</table>

<table>
<thead>
<tr>
<th>TABLE 13.3</th>
<th>Co-infections in ICK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Candida albicans and Streptococcus haemolyticus</td>
<td></td>
</tr>
<tr>
<td>2. Candida albicans and Streptococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td>3. Staphylococcus aureus and Corynebacteria</td>
<td></td>
</tr>
<tr>
<td>4. Micrococcii and Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td>5. Diphtheroids and Staphylococcus albus</td>
<td></td>
</tr>
<tr>
<td>6. Bacillus and Staphylococcus spp</td>
<td></td>
</tr>
<tr>
<td>7. Mycobacterium fortuitum and Pseudomonas aeruginosa</td>
<td></td>
</tr>
</tbody>
</table>

and Staphylococcus hemolyticus), and Candida tropicalis (Table 13.2). Other rare organisms which have been reported are Stenotrophomonas and enterococcal infections. Recurrent ICK caused by two different organisms in two successive grafts in the same patient have also been reported due to Streptococcus viridans and Candida albicans. ICK has also been reported to occur due to non-tuberculous mycobacteria after LASIK.
epithelial defects, or at a location from which loose suture has been removed or replaced.

It is characterized by the presence of insidiously progressive gray white branching opacities within the corneal stroma (Fig. 13.1). These may vary in appearance from small, round stellate opacities to lesions in which large, branching needle-like opacities extend across the entire cornea. The opacities may occur at any depth within the corneal stroma but tend to be isolated within a single lamellar plane. The overlying epithelium is often intact and, despite the infectious nature of the lesions, the eyes exhibit minimal signs of anterior segment inflammation. It may also show a multifocal presentation (Fig. 13.2).

Other rare forms of presentations include annular infiltration confluent with progressive melting and endothelial plaque formation and satellite lesions. Epithelial integrity is usually maintained but the presence of microcystic edema with marked stromal thickening may occur.

Epithelial crystalline keratopathy has also been reported which is characterized by minimal inflammation and plaque like lesion at the level of the corneal epithelium, which has fine crystalline appearance.24

In addition, ICK may also be mimicked by the deposition of calcium in the corneal stroma.18 Three cases have been described where eyes developed white crystalline anterior stromal deposits in their grafts while receiving long term topical corticosteroids.18

LABORATORY DIAGNOSIS

The causative organisms in cases of superficial lesions may be isolated from the cultures of corneal scrapings, which should be undertaken in all cases. The identification of offending organisms is generally made from cultures and histopathological analysis of corneal buttons obtained at the time of repeat corneal transplantation in most cases.

Microbiological Investigations

Corneal scrapings should be obtained and cultures should be sent for routine bacterial and fungal organisms. Routine culture methods are often unsuccessful due to the depth of the lesions, and corneal biopsies are necessary to confirm the diagnosis.

Histopathology

Light Microscopy

Characteristically intra-lamellar pockets of bacteria within the corneal stroma are isolated in a single lamellar plane with a pauci-inflammatory type of reaction. Collagen lamellae adjacent to the collections of bacteria are undisturbed with no evidence of necrosis or corneal thinning. The crystalline deposits, which are composed of bacterial aggregates, are most often in the anterior and less commonly in a mid-stromal location or in posterior stroma.
Electron Microscopy

Electron microscopy analysis of corneal specimen confirms the findings revealed on light microscopy. In addition, bacteria are often seen in varying stages of viability or degeneration. Transmission electron microscopy shows bacterial organisms with a distinct laminated cell wall. The crystalline appearance is probably due to bacterial masses between the corneal lamellae. Samples and coworkers observed electron dense bodies with needle-like projections and suggested that these may be responsible for the crystalline appearance. Amorphous material consistent with bacterial biofilm (glycocalyx) has also been observed surrounding the bacteria in these cases.

Confocal Microscopy

Confocal microscopic evaluation of patients with ICK shows, two basic patterns of crystal morphology distinct needle-like deposits at varying depths in the stroma and amorphous deposits grouped at different levels in the stroma.

Treatment

ICK responds poorly to treatment with antibiotics. It is difficult to manage a case of ICK due to the following factors:

1. Antibiotics, which interfere with cell wall synthesis, require active bacterial replication for their effect and are therefore less effective against slowly replicating...
organisms, which are usually the causative organisms of these lesions. The nutritionally variant streptococci are relatively resistant when compared to other streptococci.

2. The encasement of the bacteria with biofilms limits the bioavailability of topically administered antibiotics.

Discontinuation of the topical corticosteroids coupled with aggressive therapy with appropriate antibiotics is the mainstay of the treatment of ICK.

**Medical Therapy**

The initial treatment of ICK consists of discontinuation of topical corticosteroids therapy and intensive topical application of fortified antibiotics that are effective against the causative organisms. These include use of aqueous Penicillin G 100,000 units/mL, cefazolin sodium 50 mg/mL or vancomycin 10 to 20 mg/mL for alpha-hemolytic streptococci. These are given at 1-2 hourly frequency for 1 week and are subsequently tapered. Other antibiotics may be considered when different organisms are identified as the cause of infection.

Medical treatment has however been generally disappointing. Most cases cured by medical treatment have required prolonged use of antibiotics and visual results have been poor. In some cases prolonged use of topical antibiotics may have caused the emergence of resistant strains of organisms. Additionally, slow replication rate of organisms may impair the effectiveness of antibiotics that block cell wall synthesis.

**Surgical Therapy**

More than 50 percent of cases of ICK fail to respond to medical treatment and require therapeutic penetrating keratoplasty (Fig. 13.3). Due to minimal inflammation, the results of therapeutic keratoplasty are generally favorable.

Lamellar keratectomy in some cases has also been tried due to the infection by virulent organisms such as *Pseudomonas aeruginosa* and *Mycobacterium fortuitum*.

**Excimer Laser Ablation**

Excimer laser ablation of the infectious lesions may also be done especially if the infection is superficial. A 25 mm myopic ablation followed by 25 mm hyperopic ablation is performed using a maximum diameter of 5.8 mm.

**YAG Laser Application**

Recently YAG laser is also being used by some corneal surgeons to break the protective biofilm of microorganisms of ICK. This helps in achieving a better response to antimicrobial therapy.

**References**

Corneal infections are known to occur after various extraocular surgeries such as pterygium surgery and also after intraocular surgeries such as cataract surgery, corneal transplantation, refractive surgery, excimer laser surgery, trabeculectomy and vitreoretinal surgery.

**Keratitis after Pterygium Surgery**

Corneal ulceration is known to occur after Pterygium surgery. Many cases of Scedosporium corneoscleral infection have been seen following Pterygium surgery with beta radiation. They may be amenable to topical medications if the associated keratitis is mild or may require a large corneoscleral graft in severe cases of melting (Fig. 14.1A and B).

**Keratitis After Cataract Surgery**

Infectious keratitis can occur after cataract surgery which includes the extracapsular cataract extraction (Fig. 14.2) and the phacoemulsification surgery. The infection generally begins at the incision site which in cases of phacoemulsification may be the scleral tunnel, corneal tunnel or the side port.

The infection in the tunnels is related to the wound architecture with imperfect apposition which creates a potential space between the roof and the floor of the tunnel. In cases of the scleral tunnel, it may commence as scleritis and in cases of clear corneal incisions it manifests as keratitis. Clear corneal wound infection (Fig. 14.3) may be associated with scleritis and endophthalmitis. Simultaneous infection of the main wound and the side port has also been reported (Fig. 14.4).

**PREDISPOSING FACTORS**

The important predisposing factors in a case of wound infection include the presence of wound leak after surgery or the occurrence of infection in the adnexal areas.
The sources of microorganisms include the patient’s own eyelids and conjunctiva, contaminated instruments, lenses or irrigating solutions, airborne infections, and breaches in the sterile technique. Another important predisposing factor with cases is the use of corticosteroids without antibiotics.1,2

**CLINICAL EXAMINATION**

The diagnostic criteria in cases of keratitis following phacoemulsification surgery is the presence of an epithelial defect with or without infiltrates in the surrounding stroma along the tunnel incision, i.e., external wound, tunnel, or internal wound, and associated anterior chamber reaction (Fig. 14.3). At times, the epithelial defect may not be present. It is imperative to examine the wound, adjoining sclera, size and depth of the infiltrate, and anterior chamber reaction. In addition, Seidel’s test and irrigation of the lacrimal drainage system should also be performed to rule out wound leak and dacryocystitis, respectively.

**MICROBIOLOGIC EXAMINATION**

All patients should be subjected to a detailed microbiology workup. Specimens should be collected through corneal or scleral scraping by using a no. 15 surgical blade, corneal or scleral biopsy, and anterior chamber paracentesis. The material should be examined microscopically by using Gram stain, Giemsa stain, and potassium hydroxide and inoculated on various culture media that facilitate the growth of fungi, bacteria, and parasites (blood agar, chocolate agar, Sabouraud’s dextrose agar, non-nutrient agar, thioglycolate broth, and brain heart infusion broth).

At times scrapings from the corneal surface overlying the infiltrate may not reveal any organisms on microscopy or culture and keratitis may be present in the posterior part of the tunnel. Hence, it may be necessary to make a flap at the incision site like a trap door and biopsy may be taken from the visibly infected part of the posterior aspect of the tunnel. In cases where scleritis is present corneoscleral biopsy may be required and in cases of thick exudates associated with hypopyon anterior chamber paracentesis may be undertaken.
TREATMENT

The initial treatment should be based on the smear results and is subsequently modified after the culture report. In cases of bacterial ulcers fortified cefazolin sodium 50 mg/ml may be given with tobramycin sulphate eye drops 1.3 mg/ml every hour initially. Alternatively in cases which are not responding to treatment and in cases where resistance is seen to these drugs, vancomycin 50 mg/ml may be given. The topical antibiotics are then tapered and topical steroids such as prednisolone acetate 1% eyedrop in qid doses may be added depending on the healing response of the ulcer.

Patients who had filamentous fungal infection should be treated with topical natamycin 5 percent one hourly and cycloplegics. Additionally oral ketoconazole 400 mg/day or itraconazole 200 mg/day may be given in suspected cases of scleritis or endophthalmitis. In cases of yeast infection, topical fluconazole 2 percent or amphotericin B 0.05 percent are given hourly alongwith cycloplegics.

Microbial Keratitis After Keratoplasty

Graft infection is the most devastating complication following corneal transplant surgery and may cause graft failure if not managed promptly and appropriately (Figs 14.5A and B). The immunity of ocular surface of a corneal graft is suboptimal due to altered tear film dynamics, loss of corneal sensations and frequent instillation of topical corticosteroids in the postoperative period.

The incidence of infectious keratitis after corneal transplantation surgery varies from 1.7 to 11.9 percent in various studies.1-7 The incidence of infectious keratitis following lamellar keratoplasty has been reported to be 11.11 percent.8 Most infections present in the first year of corneal transplantation surgery.4-6

PREDISPOSING FACTORS

Various factors that can predispose to infection in a case of corneal graft include type of corneal pathology of the recipient, donor tissue contamination and ocular surface problems.

Indication for Keratoplasty

Corneal grafts performed for bullous keratopathy9 and corneo-iridic scars10 have an increased risk of keratitis as compared to corneal grafts done for keratoconus, Fuch’s endothelial dystrophy and stromal corneal dystrophies.

Donor Factors

Certain causes of donor death like malignancy, cardiac diseases and septicemia are more prone to graft infection.11 Preservative media for tissue storage may also be a potential source of graft infection due to inadvertent contamination during the processing and storage of the donor tissue.12
Recurrence of Host Infection

Recurrence of infection is usually seen when there is incomplete excision of infected tissue of the host cornea especially in cases of therapeutic grafts (Figs 14.6A and B). This occurs in cases of grafts performed for herpes simplex keratitis and may occur in 23 percent of transplanted corneas (Fig. 14.7).\(^6\)

Persistent Epithelial Defect

Persistent epithelial defect acts as a breach in the corneal protection and in such a situation there are increased chances of microbial invasion (Fig. 14.8). This has been reported to occur in 14 to 74 percent cases in various series.\(^3,6,8,10\) Lid abnormalities like trichiasis, poor quality and inadvertent damage of the donor tissue, tight suturing (Fig. 14.8), loose suturing (Fig. 14.9) and preservative toxicity can delay epithelialization leading to the occurrence of persistent epithelial defects.

Contact Lens

Bandage contact lenses, when used to promote healing of the epithelial defect may induce corneal hypoxia, reduces local immunity and can cause increased microbial adherence and a subsequent keratitis.\(^9\)
Specific Types of Keratitis

Dry Eye and Ocular Surface Problems

Ocular surface disorders like dry eye and dellen result in altered tear film dynamics and decreased tear film coating which increases the chances of adherence of the microbes to the transplanted corneas.

Suture Related Problems

Suture related problems are the most important predisposing factors for graft infection in 14 to 60 percent cases.\(^8,13-17\) Loose or exposed sutures attract mucin (Fig. 14.9) and act as a nidus for microbial invasion and proliferation and can cause graft infection. Suture abscesses may also lead to graft infection (Figs 14.10A and B). A continuous suture has a higher chance of predisposing a graft to the occurrence of infection as compared to interrupted sutures.

Systemic Associations

It has been reported that patients with diabetes mellitus may have more chances of graft infection.\(^9,10\) In developing countries graft infection is associated with low socioeconomic status of the patient.\(^10,18,19\) In our study there was 2.5 times higher chances of infection in the lower socioeconomic status as compared to higher strata and was attributed to poor living conditions and inadequate hygiene.\(^18\)

Microbiology of Graft Infection

The most common cause of graft infection is herpes simplex virus followed by bacterial organisms. More cases with Gram-positive cocci (coagulase-negative staphylococci, \textit{Streptococcus pneumoniae} and \textit{Staphylococcus aureus})\(^3,4,20\) have been reported as compared to Gram-negative organisms (\textit{Pseudomonas aeruginosa} and \textit{Serratia marcescens}). Mycotic keratitis after corneal transplantation has also been known to occur and fungi of \textit{Aspergillus} and \textit{Mucoraceae} species\(^21\) are most commonly isolated fungal organisms from these cases.

Clinical Features

Patients of graft infection in the early post-operative period present with non-specific symptoms of redness,
photophobia, foreign body sensation and purulent discharge. There may be a sudden or gradual decrease in the visual acuity depending on the location of the lesion. Infections involving the central part of cornea cause a sudden decrease in visual acuity. Some patients with infection at the graft host junction or with infection localized to the periphery of the graft may present with a normal visual acuity.

There may be a delay in diagnosis of infection in lamellar grafts, especially in the early stages, because an infiltrate may develop in the interface and hence may not be visualized earlier.8

The ulcer should be examined under slit lamp and documented with a careful detailed drawing and photographs (if possible). The size of the epithelial defect, infiltrate and hypopyon, if present should be measured and recorded at each follow-up.

The ulcers may be either peripheral or central in location. The former are usually associated with suture related problems whereas the latter are related to exposure and tear film abnormalities. Advanced cases may present with frank graft dehiscence or melting.

**Investigations**

Generally, culture swabs are taken from the donor corneoscleral rim and the media. This may aid in identification of the micro-organism especially if the infection has occurred in the early post-operative period. The corneal scraping should be done under topical anesthesia using a slit lamp biomicroscope. Smears for Gram’s stain and potassium hydroxide (KOH) wet mount should be prepared. In cases of suture related problems, the offending suture should be removed and sent for bacterial and fungal culture examinations. If a contact lens is in place, it should be removed and placed on a separate culture plate. Initially the cultures should be done on blood agar, chocolate agar and Sabouraud dextrose agar. Special culture media like Lowenstein Jensen media, non-nutrient agar with *Escherichia coli* and thioglycolate broth should be used if there is clinical suspicion of infection by unusual pathogens.

**Management**

Corneal graft infection requires a prompt and judicious management, which depends on a good clinical judgment and an early microbiological diagnosis of the ulcer.

**MEDICAL MANAGEMENT**

We prefer to hospitalize all cases with corneal graft infection. In all cases of suppurative infectious keratitis of corneal graft topical corticosteroids should be stopped immediately and an intensive regimen of broad-spectrum combination therapy with fortified cefazolin sodium 50 mg/ml and fortified tobramycin 14 mg/ml should be instituted every 30 minutes round the clock in the first 24 hours. Alternatively, fortified cefazolin 50 mg/ml and gatifloxacin 3 mg/ml may be started in the same frequency.

Antifungal medications such as 5 percent natamycin eye drops one hourly are added only if there is microbiological evidence of presence of fungus (i.e. on Gram’s smear, KOH wet mount or culture examination).

Supportive topical medical therapy is given in the form of cycloplegics, lubricants and antiglaucoma medications (if required). Topical corticosteroid may be re-started in cases of bacterial infections only if the offending organism has been identified and there is a significant positive response to the antimicrobial therapy. Systemic antibiotics are indicated in cases with frank/impending scleral involvement, graft melting, perforation or dehiscence.

The patient should be examined daily to monitor the clinical response for progression of the ulcer. Once the clinical improvement occurs, the topical medications should be tapered.

However, if the medical management fails, surgical options for the management of post-keratoplasty...
Specific Types of Keratitis

infections should be resorted to. If the cultures are negative and the keratitis worsens despite medical therapy, a diagnostic biopsy is indicated.

SURGICAL MANAGEMENT

If a loose suture is noted in the early post-operative period, it should be removed and replaced immediately, taking care that the exposed part of the suture does not traverse the corneal stroma. If a suture is the cause of infection during the late postoperative period i.e. after 3 months it should be immediately removed and sent for cultures. During the late postoperative period removal of a single interrupted suture does not adversely affect the wound stability. However, if infection occurs due to a loose continuous suture, it should be immediately removed and replaced with interrupted sutures.

The indications for a graft exchange include non-resolving graft ulcers, deep-seated abscess non responsive to treatment, wound dehiscence, perforation or graft melting. A graft exchange using the same size graft as the one used in the prior surgery may be done in cases where the infection has not spread to the host cornea or a larger sized graft may be used in cases where the infection spreads to the host cornea.

Associated Endophthalmitis

Endophthalmitis may occur early or late after penetrating keratoplasty, often with disastrous consequences. It may occur in 4 to 13 percent of the cases of graft infection.2,3,8,13 The sources of infection are contaminated donor tissue or corneal storage media or irrigating solutions. Ulcerative keratitis at the graft host junction may progress to perforation and subsequent endophthalmitis. Anterior vitrectomy performed at the time of penetrating keratoplasty may increase the chance of endophthalmitis by 1.5 times. In cases of corneal graft infection associated with endophthalmitis intravitreal injection of vancomycin 1 mg in 0.1 ml and ceftazidime 2.25 mg in 0.1 ml should be given along with topical therapy for corneal ulcer.

HERPETIC GRAFT INFECTIONS

Herpes simplex virus keratitis may recur in cases of penetrating keratoplasty performed for herpetic scars or may develop following keratoplasty without a clinical history of herpes keratitis in the host.23 The incidence of recurrence of herpetic infection after penetrating keratoplasty varies from 10 to 25 percent during the first year of follow-up.23,24

Herpetic keratitis in a corneal graft may have a variable presentation and may present as a classic dendritic ulcer (Figs 14.12A and B), persistent epithelial defect, graft rejection, or a herpetic stromal infiltration of the graft (Figs 14.12A and B).25 A geographical herpetic ulcer has to be differentiated from a neurotrophic ulcer by slit lamp biomicroscopy using Rose Bengal Staining. The risk of recurrence of herpetic infection increases specially if the corticosteroids are used after penetrating keratoplasty without the concomitant use of antiviral drugs as this enhances viral multiplication. Hence a prophylactic dose of systemic acyclovir 800 mg per day is recommended for up to year in cases of penetrating keratoplasty done for herpetic scars.24-26

Graft Survival and Visual Outcome

Visual prognosis in eyes with post-keratoplasty graft infection is poor even after a successful medical therapy due to corneal scarring after resolution of keratitis and a high rate of graft decompensation. A repeat keratoplasty is required in almost half of the cases.4,10 Clear grafts following graft infection has been reported in 23 to 67 percent cases in various studies.5,6,15,20 A best corrected visual acuity (BCVA) of better than 6/60 on Snellen’s acuity chart is seen in only 14 to 30 percent of the eyes and only 6 percent of patients achieved a best corrected visual acuity of > 6/18 at the final follow up in one study.10 Infections after lamellar keratoplasty are associated with grave prognosis and may not be amenable to antimicrobial therapy.8 This may necessitate the removal of the graft or a therapeutic penetrating keratoplasty.8

MICROBIAL KERATITIS AFTER REFRACTIVE SURGERY

Microbial keratitis has been reported after radial keratotomy, photorefractive keratectomy, laser in situ keratomileusis and laser subepithelial keratectomy.

Microbial Keratitis After LASIK

Microbial keratitis following LASIK is a rare complication but can be sight-threatening and can lead to devastating consequences. The incidence of microbial keratitis varies between 1:1000 and 1:5000.22 Most cases present within the first week after surgery; however cases have been reported even after 1 month.
Infectious keratitis following LASIK may be early onset (occurring within the first 2 weeks of surgery) or late onset (occurring 2 weeks to 3 months after surgery). The organisms seen in early-onset infectious keratitis are common bacterial pathogens such as staphylococcal streptococcal and Pseudomonas (Figs 14.3A and B) species. Gram-negative organisms are rare. The organisms seen in late-onset infectious keratitis are usually opportunistic such as fungi (Fig. 14.4), nocardia, and atypical mycobacteria.

Infectious keratitis following LASIK often presents with inflammation in the corneal interface, which can mimic diffuse lamellar keratitis (DLK). Because of this, many cases are typically treated with frequent topical corticosteroid therapy that may obscure the clinical picture with transient improvement in the inflammation. However, unlike DLK, the inflammation associated with LASIK-associated infections usually persists despite topical corticosteroids, and the underlying infections can potentially worsen with corticosteroid tapering.

The appearance of an interface inflammation more than 1 week after LASIK should be presumed to be of an infectious etiology until proven otherwise. Diffuse lamellar keratitis characteristically has a diffuse appearance, while infectious keratitis has a focal area of infiltration surrounded by diffuse inflammation.
Specific Types of Keratitis

(Figs 14.13A and B) or even focal inflammation limited to the area of the infiltrate. Any focal infiltrate surrounded by inflammation should be presumed infectious until proven otherwise.

Microbiologic Examination

Any focal infiltrate following LASIK should be considered infectious, and the practice of empirical antibiotic treatment without performing cultures of microorganisms should be avoided.

Scraping should be sent for Gram-stain, Gomori-methenamine silver stain, and Ziehl-Neelsen stain to rule out unusual pathogens such as nocardia, atypical mycobacteria, and fungi. The culture media which should be inoculated include blood agar, chocolate agar, Sabouraud’s agar, and thioglycolate broth and Lowenstein-Jensen or Middlebrook 7H-9 agar. If these special media are unavailable, blood agar may be used as atypical media. Mycobacteria grow quite well in this media also. In cases in which cultures are negative and the infection continues to worsen, a corneal biopsy or polymerase chain reaction should be contemplated.

Treatment

The topical corticosteroids should be discontinued. In cases of Post LASIK keratitis fortified cefazolin sodium 5% eyedrops along with tobramycin sulphate 1.3% eye drops are instilled hourly. In cases where there is no response to above therapy and where the flap is edematous irrigation of the flap interface with an appropriate antibiotic solution (fortified vancomycin 50 mg/mL for rapid-onset keratitis and fortified amikacin 35 mg/mL for delayed-onset keratitis) may be helpful.

In patients who work in a hospital environment, there is an added risk for methicillin-resistant Staphylococcus aureus (MRSA). In these patients, fortified vancomycin 50 mg/mL may be given instead of cefazolin every 30 minutes to provide more effective therapy against MRSA. In addition, oral doxycycline 100 mg twice a day may be used to inhibit collagenase production.

For delayed-onset keratitis, which is commonly due to atypical mycobacteria,22 nocardia, and fungi, therapy should be commenced with amikacin 35 mg/mL every hour, alternating with a fourth-generation fluoroquinolone (gatifloxacin 0.3% or moxifloxacin 0.5%). Clarithromycin 1 percent and Oral Clarithromycin may also be tried in cases which do not respond to amikacin. Topical medications are generally given for 4 months and systemic medications are given for 2 months.

In cases where total melting of the cornea occurs despite amputation of the flap and maximal medical therapy, a therapeutic keratoplasty may be required to save the eye.

References

**Introduction**

Endophthalmitis is secondary to infectious keratitis although rare is a serious ocular infection that can result in blindness. Keratitis with endophthalmitis has been reported in 6.3 percent of cases. Considerable vitreous inflammation can occur with microbial keratitis in the absence of histologically demonstrable micro-organisms in the vitreous. Microbial keratitis associated with suppurative endophthalmitis is usually caused by virulent (coagulase-negative staphylococci, streptococci, and Gram-negative organisms) organisms.

**Pathogenesis**

Once endophthalmitis occurs, damage to ocular tissues occurs due to direct effect of microbial replication as well as initiation of a fulminant cascade of inflammatory mediators. Endotoxins cause direct cellular injury and cytokines attract neutrophils which enhance the inflammatory effect.

**Predisposing Factors**

Patients in whom keratitis is associated with endophthalmitis give a history of frequent corticosteroid use. They may also have a concomitant systemic disorder associated with relative immune dysfunction. Patients in whom there is absence of an intact posterior capsule, presence of wound abnormalities (in post surgical cases), or occurrence of corneal perforation are also at a greater risk of having endophthalmitis.

**Clinical Features**

**SYMPTOMS**

Most patients complain of a sudden onset and a rapid worsening of pain accompanied by a significant decrease in vision. Other symptoms that may be present are discharge, excessive tearing, increased sensitivity to normal light (photophobia), increase in redness of the eye and blepharospasm (Table 15.1).

**SIGNS**

On examination, the visual acuity is decreased and may be hand motions or only light perception in fulminant cases. There is marked conjunctival hyperemia, chemosis and circumcorneal congestion. There is presence of a corneal ulcer and the adjacent cornea is grossly edematous. A limbal ring abscess or corneal melting may also be present. The underlying findings in the anterior chamber and the posterior segment are not visible due to the presence of the overlying keratitis. The anterior chamber shows a significant degree of flare and cells, the reaction sometimes being frankly fibrinous. The iris pattern is lost, appears muddy and boggy and is resistant to dilation. There may be presence of posterior synechiae. Pupillary response to light is absent or sluggish. In more severe cases, a dense discrete or confluent, yellowish vitreous exudation is evident (Figs 15.1 and 15.2). The intraocular pressure may be elevated in the early stages of endophthalmitis. The fundus glow may be dull or absent.

**Investigations**

The investigations in a case of keratitis with endophthalmitis include clinical investigations such as ultrasonography and microbiological evaluation.
ULTRASONOGRAPHY

The presence of a corneal ulcer prevents the proper assessment of the posterior segment and hence ultrasonography A and B scan in these cases is mandatory to detect the involvement of vitreous and for monitoring the course of infective process.\(^5\)

In a patient with suspected endophthalmitis where retinal details are not visible it is mandatory to undertake ultrasonography whenever it is available, before instituting any form of invasive, diagnostic or therapeutic interventions. This is to detect the presence of a choroidal detachment, retinal detachment, the degree of vitreous exudation and the presence of posterior vitreous detachment. Ultrasonography is a useful aid in establishing the diagnosis, prognostication, of the case planning the surgery and sometimes in follow-up.

CORNEAL SCRAPINGS

Corneal scrapings should be obtained as is done in a routine case of corneal ulcer. The scrapings should be sent for smear (Grams, Giemsa and KOH wet mount preparation) as well as culture examination (Blood Agar, Chocolate Agar and Sabrouad’s dextrose agar).

AQUEOUS AND VITREOUS TAP

The most important samples to culture are aspirates from the aqueous and vitreous cavity. The possibility of isolating an organism in vitreous tap is 56-70 percent and in aqueous sample is 36-40 percent Hence it is necessary to culture specimens obtained from both aqueous and vitreous samples.

Aqueous Tap

Aqueous tap is obtained by a paracentesis using a 25-27 gauge, half inch needle mounted on a tuberculin syringe with its plunger on. About 0.1ml of fluid is aspirated in a controlled manner by gently withdrawing the plunger. The needle may be directly inoculated into the culture media. A part of the aspirate is ideally plated directly on to the culture media while any remaining aspirate is used to prepare slides for Gram stain and Giemsa stain.

Vitreous Tap

If there is no underlying rhegmatogenous retinal detachment, an intravitreal antibiotic injection (Vancomycin 1 mg in 0.1 ml with Ceftazidime 2.25 mg in 0.1 ml) is the treatment of choice in cases with bacterial endophthalmitis.

In cases of fungal endophthalmitis 10 μg of amphotericin B is given intravitreally.

Our technique for performing vitreous tap and giving intravitreal antibiotics is as follows:

1. Place a drop of topical anesthesia (0.5% proparacaine) on the affected eye.
2. Culture the conjunctival surface with a conjunctival swab.
3. Prepare the globe with a 5 percent povidone iodine (Betadine) solution. Insert a sterile lid speculum between the lids.
4. Perform a vitreous tap with a 23-gauge needle on a tuberculin (TB) syringe, removing 0.2 ml of vitreous for cultures.

5. Choose an injection site 3-4 mm posterior to the limbus, depending on the lenticular status. To anesthetize this site, hold an anesthetic-soaked cotton swab at the site for one to two minutes. If the patient is extremely uncomfortable, as is often the case, use a subconjunctival injection of lidocaine in addition to the topical anesthesia.

6. Enter the globe at the injection site, using a ½ inch 26-gauge needle on a TB syringe. The needle should point towards the optic nerve. Avoid entering deeper than 1 cm, ensuring entry into vitreous cavity and not in the suprachoroidal space.

7. Inject the antibiotic, and then withdraw the needle. Re-enter the globe in the same site with each of the two intravitreal antibiotics in TB syringes with 30-gauge needles. Inject 0.1 cc of each antibiotic slowly and separately. The bevel of the needle should be facing anteriorly, in order to minimize contact between the drug and the macula. In suspected cases of fungal endophthalmitis Amphotericin B is injected.

8. Remove the lid speculum. Measure the intraocular pressure after the procedure.

The inocula from the AC tap as well as the vitreous tap ideal is plated on the following:

1. Blood agar plate at 37 degree C
2. Chocolate agar plate at 37 degree C in a 4-10 percent CO₂ enriched environment
3. Sabourauds medium without any inhibitors at room temperature
4. Thioglycolate broth at 37 degree C
5. Two clean glass slides for Gram and Giemsa stain
6. Brain-heart infusion or cooked meat broth.

**TREATMENT**

The treatment of keratitis with endophthalmitis consists of medical and surgical therapy.

**Topical Therapy**

The medical therapy consists of the treatment of corneal ulcer as described previously using a combination of fortified antibiotics in frequent doses. Topical fortified cefazolin sodium 5% along with tobramycin sulphate 1.3% are given in hourly dosage for the initial 48 hours. Topical cycloplegics such as homatropine eye drops 2% is given thrice a day. In the event of raised intraocular pressure, anti-glaucoma medications such as timolol maleate 0.5% is given twice a day. Topical steroids are withheld till the corneal healing begins.

**Intravitreal Antibiotics**

Intravitreal injection of antibiotics (i.e vancomycin 1 mg in 0.1 ml with ceftazidine 2.25 mg in 0.1 ml) is given in cases of bacterial endophthalmitis, following which response is monitored until 36 to 72 hours. In case there is no response, a repeat injection may be given again. Likewise following intravitreal injection of antifungals (i.e. 10 μg of amphotericin B) in cases of fungal endophthalmitis, one waits for one week and a repeat injection of amphotericin B may be given if there is no response.

**Systemic Therapy**

We recommend the routine use of systemic steroids in all cases except when contraindicated systemically or due to fungal infections. Tab prednisolone 1.5 mg/kg body weight is given for the initial 2 weeks which is tapered after the inflammation decreases. Systemic steroids are helpful in controlling and inhibiting the inflammatory effects of endotoxins and subsequent optic nerve damage.

Systemic antibiotics consists of intravenous vancomycin 1 gm bd, intravenous amikacin or gentamicin 80 mg tds and intravenous metronidazole 500 mg tds (in case where anaerobic infections are suspected) which are given in cases of bacterial endophthalmitis. Systemic antifungal agents consist of use of oral ketoconazole 600 mg per day. Fluconazole 200 mg bd or more recently voriconazole 100 mg bd may be given in recalcitrant cases. Liver function test have to be repeated after every 2 weeks in these cases.

The patients should be examined twice daily during the initial treatment period to assess adequate sterilization of the vitreous cavity, control of intraocular inflammation, and identification of the need for additional intervention.

The overlying corneal ulcer and edema prevents immediate surgical intervention for the vitreous infection.

**SURGICAL THERAPY**

Earlier, the surgical intervention was not carried out in cases of endophthalmitis associated with corneal ulcer
due to poor prognosis. More recently, a trial of vitreous surgery (vitrectomy) may be given in which one of the temporary kerato-prostheses is used. A team approach of an anterior segment and posterior segment surgeon is required. Though the results are not as good as in cases with either corneal ulcer or endophthalmitis alone, yet the patient may obtain ambulatory vision.

An early vitreous surgery should be planned in cases with endophthalmitis and keratitis before the corneal involvement becomes so extensive so as to prevent the feasibility of vitreous surgery. This occurs as a temporary keratoprosthesis may not hold in place due to corneal melting. The vitreous surgery can usually be performed if the corneal involvement is not complete. Sometimes, it is required to perform a near complete vitrectomy especially when an associated retinal detachment is present. In such cases the vitreous surgery is done, internal subretinal fluid drainage and silicone oil tamponade is given to flatten the retina. A 360° retinal endophotocoagulation is usually not possible (because of overlying corneal involvement) and should not be performed, as the retina is inflamed and therefore more likely to develop secondary retinal tears. In such cases, if the patient recovers from the disease process and has visual potential, a 360° retinopexy by cryotherapy in a phased gradual manner can be done over 2-3 weeks following which removal of silicone oil is done.

References

Neurotrophic Keratitis

Introduction

Neurotrophic keratitis involves various degrees of degenerative corneal and conjunctival changes secondary to loss of sensory function in the pathway of the nasociliary branch of the trigeminal nerve with or without decreased tear production. The characteristic features of neurotrophic keratopathy includes breakdown of the corneal epithelium in the absence of desiccation, infection, or trauma. This persistent epithelial defect if left untreated extends into the deeper layers of the stroma and may result in corneal perforation and melting.

Etiology

The neurotrophic keratitis may occur due to ocular factors, systemic factors or a combination of the ocular and the systemic factors (Table 16.1).

**OCULAR CAUSES**

The most common ocular cause of occurrence of neurotrophic keratitis is herpes virus infection. Both herpes zoster ophthalmicus and herpes simplex keratitis can cause loss of corneal sensations subsequent to loss of corneal sensations is herpes virus infection of the ocular surface. Neurotrophic ulcers are seen in up to 25 percent of the patients with herpes zoster ophthalmicus. While herpes zoster ophthalmicus involves the ophthalmic division of the trigeminal nerve, the herpes simplex infection of the cornea effects the terminal distribution of the trigeminal nerve Other causes include corneal dystrophies, local trauma to the corneal nerves and topical medications. The topical anesthetic agents may also cause neurotrophic keratitis when these agents are used for relief of eye pain.

**SYSTEMIC CAUSES**

Systemic disorders can also cause damage to the trigeminal nerve and reduce corneal sensations. The most common systemic causes of neurotrophic keratitis include tumors and surgeries, which may damage the trigeminal nucleus, root, ganglion or any segment of ophthalmic branch of the trigeminal nerve. This

**TABLE 16.1**

<table>
<thead>
<tr>
<th>Causes of decreased corneal sensations</th>
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</thead>
<tbody>
<tr>
<td><strong>Ocular Causes</strong></td>
</tr>
<tr>
<td>Herpes Zoster Ophthalmicus</td>
</tr>
<tr>
<td>Herpes Simplex Keratitis</td>
</tr>
<tr>
<td>Lyme Disease</td>
</tr>
<tr>
<td>Contact Lens Wear</td>
</tr>
<tr>
<td>Trauma to ciliary nerves by diathermy, laser, cryotherapy or scleral buckling, corneal surgery, cataract surgery, lamellar keratoplasty, penetrating keratoplasty, refractive surgery, epikeratophakia</td>
</tr>
<tr>
<td>Topical drugs e.g. anesthetic agents, Timolol maleate (usually temporary), Betaxolol, Sulfacetamide 30 percent, Atropine, Diclofenac sodium</td>
</tr>
<tr>
<td>Ocular surface toxicity e.g. chemical burns, carbon disulfide exposure, hydrogen sulfide.</td>
</tr>
<tr>
<td>Corneal dystrophies e.g. lattice, granular (rare)</td>
</tr>
<tr>
<td>Adie’s syndrome</td>
</tr>
<tr>
<td><strong>Systemic causes</strong></td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Leprosy</td>
</tr>
<tr>
<td>Vitamin A deficiency</td>
</tr>
<tr>
<td><strong>Fifth Nerve palsy</strong></td>
</tr>
<tr>
<td>Trigeminal nerve palsy e.g. surgical (as for trigeminal neuralgia) aneurysm, tumor (e.g. acoustic neuroma) facial trauma</td>
</tr>
<tr>
<td>Congenital causes, e.g Familial dysautonomia, Goldenhar’s syndrome, Mobius syndrome, Parry-Romberg syndrome, Bassen-Kornzweig syndrome</td>
</tr>
<tr>
<td><strong>Toxins</strong></td>
</tr>
<tr>
<td>Carbon di sulfide exposure</td>
</tr>
<tr>
<td>Hydrogen sulfide exposure</td>
</tr>
</tbody>
</table>
Neurotrophic Keratitis specially occurs during any ablative procedure for trigeminal neuralgia. Following resection of the acoustic neuroma, both the fifth and the seventh nerves may be damaged.

Diabetics may have generalized peripheral neuropathy including that of trigeminal nerve especially in long standing cases and a pan-retinal photocoagulation may deepen the corneal hypesthesia already present. Further, these patients may require surgical intervention for proliferative vitreo-retinopathy and vitreous hemorrhage and are at a risk of developing persistent epithelial defects postoperatively.

Leprosy is also known to cause corneal hypesthesia (8.1 to 59.2% of cases) and neurotrophic keratitis.

PATHOGENESIS

The corneal epithelial proliferation is regulated by the sensory and the sympathetic nerves and their neurotransmitters. These sensory neurons directly affect the integrity of the corneal epithelial cell mitosis and cause an epithelial defect. This defect occurs centrally as the corneal epithelium is persistently regenerated centrifugally from the periphery and sheds old cells at its apex as described by Thoft “X, Y, Z hypothesis.”

Corneal anesthesia due to lack of afferent limb of the reflex, also cause reduction in reflex tearing and blinking. Tear mucus secretions increase and abnormalities of corneal epithelial surface makes it more prone to injury.

Diagnosis

Determination of the primary etiology of neurotrophic keratopathy is extremely important. The history of the present illness should include a thorough medical and surgical workup of the patient.

HISTORY

Patients with neurotrophic keratitis are usually asymptomatic and may only complain of blurring of vision. A detailed ocular history should include prior episodes of recurrent pain, watering and presence of blisters on the face or the eyelids.

One should also enquire regarding the prior history of any ocular trauma (chemical burns) or any other ocular pathology. Any history of use of contact lens or ocular surgery such as cryotherapy, diathermy, cataract or corneal surgery should be taken. The patients should also be enquired about the use of topical NSAIDs (Non-steroidal anti-inflammatory drugs) and topical anesthetic agents which may predispose an eye to neurotrophic keratitis.

A detailed systemic history should include history of diabetes mellitus, leprosy, or history of any surgery or facial/ head trauma or tumors.

OCULAR EXAMINATION

A detailed external examination as well as slit lamp biomicroscopy should be done. The eyelids should be examined carefully for any defects, scarring, ectropion or lagophthalmos.

Absence of nasal lacrimal tearing reflex along with ipsilateral loss of sensations in the nasal mucosa is a high risk for the occurrence of neurotrophic corneal ulceration.

Slit-lamp Biomicroscopy

The tear film should be examined carefully. Mackie has classified the characteristic stages of neurotrophic keratitis as follows (Table 16.2):

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Rose Bengal staining of the palpebral conjunctiva Decreased tear break-up time Increased viscosity of tear mucus Punctate epithelial staining with fluorescein Scattered small facets of dried epithelium (Gaule spots)</td>
</tr>
<tr>
<td>Stage II</td>
<td>Acute loss of epithelium, usually under the upper lid Surrounding rim of loose epithelium Stromal edema Aqueous cell and flare Edges of the defect become smooth and rolled with time</td>
</tr>
<tr>
<td>Stage III</td>
<td>Stromal lysis, sometimes resulting in corneal perforation</td>
</tr>
</tbody>
</table>


TABLE 16.2 Clinical stages of neurotrophic keratitis
Specific Types of Keratitis

Palpebral conjunctival surface. The tear break up time is also decreased and the viscosity of the tears increases. Punctate epithelial staining with fluorescein may also be present. Small facets or depressions of drying epithelium also known as Gaule spots may be seen on retro-illumination.

Stage II

In this stage the superficial punctate defects, which may progress to large epithelial defects surrounded by a rim of loose epithelium. Gradually the edges of the epithelium get rolled up with surrounding stromal edema. If allowed to progress further, a neurotrophic ulcer develops with characteristic features of a punched-out ulcer (Figs 16.1 and 16.2). Such an ulcer is usually horizontally oval or circular in shape with thickened borders formed by heaped up epithelial cells. Due to stromal edema, descemet’s folds may develop and this may be accompanied by flare and cells in the anterior chamber. This may or may not be associated with a sterile hypopyon. Following this, collagenase enzymes start acting and lead to stromal lysis, necrosis, and perforation. Secondary infection may occur at any stage of development of the ulcer. The ulcer usually heals with epithelial hyperplasia and irregularity, stromal scarring, and neovascularization.

Stage III

Stromal lysis is the feature of this stage and may lead to stromal necrosis, perforation and secondary bacterial infection.

Corneal Sensations

Corneal sensations are checked for the pattern and degree of corneal anesthesia gives a clue regarding local or systemic pathology. Patchy loss of corneal sensation indicates a local condition such as herpes zoster keratitis. Segmental evaluation of corneal sensation is therefore important compared to a simple measurement of central corneal sensation. Prominence and beading of the corneal nerves may be a subtle sign of lepromatous corneal involvement. Decreased corneal sensations may also occur in advanced lattice and granular dystrophies.

Corneal sensations are checked for the pattern (four quadrants and central area) and degree. Following two methods are employed to test the corneal sensations:

Cotton Wisp Method

This technique is performed using the slit-lamp biomicroscope. A cotton wisp is lightly touched first at the central cornea and then at different quadrants. Sensitivity varies in the different parts of the cornea, the central area and horizontal meridian being the least sensitive. If the touching of the cotton wisp incites a blink reflex and is felt by the patient, corneal sensitivity is graded as normal. If the corneal blink reflex is absent and forceful application is required for subjective sensation, corneal sensitivity is graded as diminished. An absent blink reflex and the absence of subjective touch sensation to the cotton wisp application is interpreted as complete corneal anesthesia.
**Cochet and Bonnet Aesthesiometer**

This instrument is more accurate than the cotton wisp method and helps in the quantitative assessment of the pattern and severity of corneal anesthesia.\(^{13}\) It consists of a standardized nylon filament 0.12 mm in diameter contained in a calibrated holder (Fig. 16.3). For the quantitative measurement of corneal sensations, the length of the monofilament is changed according to the scale reading (60.5 mm) along the holder. A conversion table is provided with the instrument, which converts the length of the filament into pressure in grams per square millimeter (11 mg to 200 mg/0.0113 mm\(^2\)) (Table 16.3).

The severity of corneal hyposensitivity may be graded as follows: A mean corneal sensitivity of 55 mm or more, measured in five separate quadrants of the cornea is considered normal corneal sensitivity. A value of 50-54 mm indicates severe hyposensitivity, and a value of 30 mm or lower depicts advanced corneal hyposensitivity. This test helps us to define those patients who are at a risk for development of neurotrophic keratopathy.

Associated ocular findings may help to elicit the etiology of corneal hypesthesia. Iris atrophy with or without anterior chamber reaction is present with herpes zoster and simplex keratouveitis and leprosy.

**Dilated Fundus Examination**

Dilated fundus examination should be done in all cases as optic nerve swelling or pallor may indicate an orbital or retro-orbital lesion. Diabetic retinopathy could indicate the likelihood of diabetic neuropathy and laser scars from pan-retinal photocoagulation, which may indicate ciliary nerve damage and hence decreased corneal sensations.

**TABLE 16.3**

<table>
<thead>
<tr>
<th>Nylon length (mm)</th>
<th>60</th>
<th>55</th>
<th>50</th>
<th>45</th>
<th>40</th>
<th>35</th>
<th>30</th>
<th>25</th>
<th>20</th>
<th>15</th>
<th>10</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value of pressure in mm Hg</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>16</td>
<td>21</td>
<td>27</td>
<td>36</td>
<td>52</td>
<td>75</td>
<td>100</td>
<td>145</td>
<td>200</td>
</tr>
<tr>
<td>Mean value of pressure in gm/sq/mm</td>
<td>0.96</td>
<td>1.08</td>
<td>1.16</td>
<td>1.4</td>
<td>1.84</td>
<td>2.4</td>
<td>3.2</td>
<td>4.6</td>
<td>6.64</td>
<td>8.84</td>
<td>12.84</td>
<td>17.68</td>
</tr>
</tbody>
</table>

**Systemic Examination**

Systemic examination, especially neurological examination, is of particular importance. The presence of other neurologic deficits helps to localize the neoplasms, injuries, vascular accidents that may include the fifth cranial nerve or its brain stem nucleus. Seventh and eighth nerves may be involved especially in acoustic neuromas. Ocular motility may also indicate malfunctioning of the cranial nerves III, IV and VI, which may localize an aneurysm or cavernous sinus pathology.

Papillary abnormalities are related to the dysfunction of the cranial nerve II and defects in the sympathetic innervation of the iris. The presence of an afferent papillary defect with corneal hypesthesia indicates the intraconal lesion of the orbit. Abnormal pupil reactions associated with Adie’s pupil is a cause of neurotrophic keratitis.

Sensations of the skin and the nasal mucosa should also be checked. Immunological and connective tissue system work-up should also be performed and thyroid ophthalmopathy should also be ruled out.

**Differential Diagnosis**

Neurotrophic keratitis must be differentiated from herpetic epithelial keratitis (Table 16.4). The latter may occur at any site and is due to active viral infection. It usually presents as a dendritic ulcer with branching pattern and terminal bulbs with swollen epithelium at the borders. However, a geographic ulcer may mimic neurotrophic keratitis.

The neurotrophic keratitis on the other hand, is seen most often in the interpalpebral area. It presents as a persistent epithelial defect with boggy epithelium and may have sometimes a dendritic shape. A classical neurotrophic ulcer is usually smooth, typically round or oval in shape with heaped epithelium at the border.

Rose Bengal staining may conclusively help in making a clinical diagnosis. In herpetic corneal ulceration the epithelial cells present at the margins are dead and stain heavily. In neurotrophic keratitis, the...
epithelial cells at the margin are healthy and do not stain with Rose Bengal.

**Investigations**

The investigations in a case of neurotrophic keratitis should include ocular and systemic investigations. Although neurotrophic ulcers are sterile by definition, this should not be presumed clinically. Bacterial super infections are common and an active herpes keratitis may simulate a neurotrophic ulcer. If a super-infection is suspected in a patient with a neurotrophic ulcer, scrapings should be taken from the leading edge and base of the ulcer and sent for culture and sensitivity testing before initiation of therapy. The specimen obtained from the scraping should be directly inoculated onto blood agar, chocolate agar, Sabouraud agar plate, and viral culture medium. Multiple smears should be made for examination under Gram’s, Giemsa, and Grocott’s methenamine silver stain. Direct immunofluorescence staining should be done for herpes virus.

A magnetic resonance imaging of the brain and orbits should be done when neurotrophic keratitis is associated neurologic deficit or the etiology of corneal hypesthesia is in doubt.12

**Treatment**

Selection of the treatment modality depends on the stage of the ulcer which is being treated.

<table>
<thead>
<tr>
<th>STAGE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>The treatment of Stage I of neurotrophic keratitis consists of withdrawal of all epitheliotoxic drugs, use of ocular lubricants (preferably preservative free) and bandage soft contact lenses. In some cases, one may also consider the application of the punctual plugs, if the tears are deficient. Oral tetracycline 250 mg twice a day or doxycycline 100 mg every other day may be used as it reduces the amount of mucus produced and may be used as an adjunct to above therapy.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STAGE II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocular Lubricants</strong></td>
</tr>
<tr>
<td>Just like in stage 1, copious preservative free artificial tears and ointment should be used to protect the vulnerable epithelium until it adheres tightly to the stroma.</td>
</tr>
</tbody>
</table>

| **Growth Factors and Cell Attachment Factors** |
| There are various reports available on the use of various growth factors and cell attachment factors, which stimulate the proliferation and differentiation of corneal epithelial cells and prevent recurrent epithelial erosions. The various growth factors, which have been used, include nerve growth factor,21 epidermal growth factor,22 laminin23 Fibronectin and albumin.24  |

| **Transpore Tape or Patching** |
| Eyelid closure with transpore tape or pressure patching may be done which is a temporary measure. |

| **Tarsorrhaphy** |
| Lateral or a complete tarsorrhaphy should be performed to protect loosely attached or non-adherent regenerating epithelium from the windshield wiper action of the eyelids. Tarsorrhaphy is an easy and reversible process and is preferred in patients who are bedridden or those who require lid closure for short duration. In many cases, tarsorrhaphy may be maintained for 1 year as the premature opening of tarsorrhaphy may result in recurrence of corneal epithelial disruption. |

| STAGE III |
| Despite every effort, the corneal epithelial defect may progress to corneal ulcer with stromal lysis resulting in |
corneal thinning and perforation. During this stage it is important to maintain the integrity of the corneal surface.

**Collagenase Inhibitors**

Proteolytic enzymes play a major role in the formation and progression of ulcer. Topical cysteine, acetylcysteine 20 percent, ethylene-diaminetetra-acetic acid 0.2 mol/L, or tetracycline inhibit collagenase activity and decrease stromal meltdown. These collagenase inhibitors are relatively nontoxic, although their efficacy in humans has not yet been conclusively demonstrated.

Tetracycline has also been reported to protect the cornea against proteolytic degradation after chemical burns by inhibiting matrix metalloproteinases (MMPs). Initially it was believed that tetracycline acts by inhibiting MMPs after binding with cations, i.e. Zn and Ca but it is now thought to act primarily through restriction of the gene expression of neutrophil collagenase and epithelial gelatinase. Tetracycline is given in the dose of 250 mg 4 times a day or doxycycline 100 mg twice a day for 4 to 6 weeks.

**Tissue Adhesive**

Tissue adhesive, particularly isobutyl cyanoacrylate (histoacryl) along with a bandage contact lens is used as an adjunctive treatment for filling the corneal ulcer and perforation (< 2 mm) and providing tectonic support to the cornea. Tissue adhesives also aid in the following:

1. Exclusion of polymorphonuclear leukocytes from the involved stroma (possibly by creating a hypoxic environment which is not favorable to polymorphonuclear leukocytes)
2. Antibacterial action

**Figures 16.4A to D:** Neurotrophic keratitis healed after multi-layered amniotic membrane transplantation
3. Promotion of neovascularization and postponing keratoplasty in an acutely inflamed eye.

**Conjunctival Flap**

Despite the prompt use of non-surgical modalities, the ulcer may progress, requiring surgical intervention. A conjunctival flap halts the inflammatory process, eliminates frequent medications, improves cosmesis, and provides an alternative to invasive surgery or enucleation. Several studies have been done on the use of the conjunctival flap for persistent ocular surface disease and its role is well established.

A total conjunctival flap or partial or bridge conjunctival flap is indicated to prevent progression of the ulcer to perforation. Total conjunctival flap (Gunderson flap) is indicated for patients with extensive stromal destruction with a poor visual prognosis. For a peripheral or small ulcer, a partial or bridge conjunctival flap, particularly of vertical orientation, is performed. Along with structural support, the partial conjunctival flap maintains good vision for the patient by not involving the central area.

**Amniotic Membrane Transplantation**

Multi-layered amniotic membrane is used for filling the cavity of the ulcer and restoring the stromal thickness and integrity of the corneal epithelium. For this procedure cryo-preserved amniotic membrane is employed. Small pieces are cut from the membrane and carefully placed into the base of the ulcer. Depending on the depth and the configuration of the ulcer, two or more of these pieces are stacked one above the other to...
fill the cavity of the ulcer (Figs 16.4A to 16.5D). Finally, a larger piece of membrane is trimmed to cover the ulcer and the de-epithelialized zone surrounding the ulcer. The membrane is then secured to maintain its physiologic orientation (epithelium up and stroma facing the ulcer) with six or more interrupted 10-0 nylon sutures. The knots of the sutures are cut short but not buried. On completion of the surgery, the eye is covered by a soft bandage contact lens.

The advantages of using a multi-layered amniotic membrane are:

1. Stromal thickness is maintained even after the amniotic membrane dissolves (perhaps because the amniotic membrane modifies the proliferative and migratory behavior of stromal keratocytes).
2. It allows rapid epithelial wound healing and long-term stability of the corneal surface.
3. It provides an effective barrier for inflammatory cell entry.
4. It downregulates the synthesis of chemotactic factors and thereby reduces corneal inflammation.

**Penetrating Keratoplasty**

For large corneal perforations (>2 mm) an emergency small lamellar (blow-out) or full thickness graft is required in an acutely inflamed eye. However, grafts do poorly in such anesthetic corneas.

**References**

Peripheral Ulcerative Keratitis

Introduction
Peripheral corneal ulceration or peripheral ulcerative keratitis (PUK) is usually characterized by crescent-shaped destructive inflammation of the juxtalimbal corneal stroma which is associated with an epithelial defect, presence of stromal inflammatory cells, and progressive stromal degradation and thinning. Conjunctival, episcleral and scleral inflammation are usually present. It leads to progressive necrosis of the corneal stroma, leading to perforation and blindness.

Epidemiology
Collagen vascular diseases are responsible for approximately half of the non-infectious cases of PUK. Rheumatoid arthritis (RA) is the most common collagen vascular disorder that causes PUK and is responsible for 34 percent of cases of noninfectious PUK. It may also be the initial manifestation of Wegener’s granulomatosis (WG) and polyarteritis nodosa (PAN).

It is rarely reported after relapsing polychondritis (RP) and systemic lupus erythematosus (SLE). Mooren’s ulcer is a rare local autoimmune disease associated with PUK and is a diagnosis made by exclusion. It is believed that some of these cases may have been the presenting manifestation of an occult systemic disease rather than a true Mooren’s ulcer.

There is no predilection for any age for a case of PUK. Since PUK is more common in people with collagen vascular disorders (especially RA), it is more common in females than in males. However, Mooren’s ulcer is more common in males than females.

Etiology
First and foremost, an infectious origin of the PUK must be excluded. In any case of limbal thinning associated with corneal infiltrate, it is mandatory to assume an infectious etiology until proved otherwise.

Although most bacterial organisms affect the central cornea, all are capable of producing limbal ulceration. The most likely microbial organisms, which can cause PUK, include Staphylococcus (Fig. 17.1) fungal organisms (Fig. 17.2), and herpes simplex virus (Figs 17.3A and B).

The major risk factors of PUK are the connective tissue and vasculitic diseases (Table 17.1). Other disorders that can cause PUK include systemic and local infectious conditions, as well as local degenerative disorders (Table 17.1).

Pathogenesis
The peripheral cornea has distinct morphologic and immunologic characteristics, which make it prone to inflammatory reactions. Unlike the avascular central cornea, the peripheral cornea is closer to limbal conjunctiva and derives its blood supply and lymphatics from the limbal capillary vessels, which extend approximately 0.5 mm into the clear cornea. This is a source of immunocompetent cells, such as macrophages, Langerhan’s cells, lymphocytes, and plasma cells. Immune complexes circulating in the blood of the patients with collagen vascular disease can lodge at the ends of the limbal vessels by virtue of their size and the restrictive molecular sieving characteristics of the cornea. The peripheral cornea contains five times higher levels of the first component of the complement (C1) than does the central cornea which stimulates chemotaxis of the neutrophils to the area. The concentration of Ig M which is a large molecule directed against Ig G (rheumatoid factor) is also greater at this site. Immune complexes of rheumatoid factor and Ig G can deposit in the peripheral cornea and produce inflammation and corneal ulceration. Langerhans’ cells, which are the pre-eminent antigen-presenting cell, are also concentrated more densely in the peripheral cornea.
Any inflammatory stimulus in the peripheral cornea that is caused by invasion of microbial organisms (bacteria, virus, fungi, and parasites), immune complex deposition (in systemic immune diseases), trauma, malignancy, or dermatologic conditions may produce local and systemic immune responses, resulting in neutrophil recruitment and complement activation at the limbus.

Activated complement components increase vascular permeability and further generate chemotactic factors for neutrophils (e.g. C3a, C5a), which infiltrate the peripheral cornea and release proteolytic and collagenolytic enzymes, reactive oxygen metabolites, and proinflammatory substances (such as platelet-activating factor, leukotrienes, prostaglandins), causing dissolution and degradation of the corneal stroma. The inflamed limbal conjunctiva itself is capable of producing collagenase, which also causes stromal degradation.

Systemic diseases that may cause immune complex deposition at the peripheral cornea and cause PUK include collagen vascular diseases such as rheumatoid arthritis (RA), Wegener’s granulomatosis (WG), polyarteritis nodosa (PAN), relapsing polychondritis (RP), and systemic lupus erythematosus (SLE). Infectious conditions, whether systemic (hepatitis, syphilis) or local (herpes simplex keratitis, fungal keratitis), and noninfectious local disorders (Mooren’s ulcer, marginal keratitis) also may cause PUK.
Specific Types of Keratitis

**TABLE 17.1**
Etiology of peripheral ulcerative keratitis

<table>
<thead>
<tr>
<th>Infections</th>
<th>Non-infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocular</strong></td>
<td></td>
</tr>
<tr>
<td>• Bacterial (Staphylococcus, Streptococcus, Moraxella, Haemophilus Gonococcus)</td>
<td>• Mooren’s ulcer</td>
</tr>
<tr>
<td>• Viral (Herpes simplex, herpes zoster)</td>
<td>• Terrien’s marginal degeneration</td>
</tr>
<tr>
<td>• Acanthamoeba</td>
<td>• Pellucid marginal degeneration</td>
</tr>
<tr>
<td>• Fungal organisms</td>
<td>• Blepharitis</td>
</tr>
<tr>
<td></td>
<td>• Keratoconjunctivitis sicca</td>
</tr>
<tr>
<td></td>
<td>• Neurotrophic and neuroprotective</td>
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<tr>
<td></td>
<td>• Nutritional deficiency</td>
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<tr>
<td></td>
<td>• Ocular chemical injury</td>
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<tr>
<td></td>
<td>• Contact lens</td>
</tr>
<tr>
<td></td>
<td>• Trauma</td>
</tr>
<tr>
<td></td>
<td>• Post-surgical</td>
</tr>
<tr>
<td><strong>Systemic</strong></td>
<td>• Rheumatoid arthritis</td>
</tr>
<tr>
<td>• Tuberculosis</td>
<td>• Giant cell arthritis</td>
</tr>
<tr>
<td>• Syphilis</td>
<td>• Wegener’s granulomatosis</td>
</tr>
<tr>
<td>• Varicella zoster</td>
<td>• Systemic lupus erythematosus</td>
</tr>
<tr>
<td>• Gonorrhea</td>
<td>• Sjögren’s syndrome</td>
</tr>
<tr>
<td></td>
<td>• Relapsing polychondritis</td>
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<tr>
<td></td>
<td>• Progressive systemic sclerosis</td>
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<tr>
<td></td>
<td>• Churg-Strauss syndrome</td>
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<tr>
<td></td>
<td>• Crohn’s disease</td>
</tr>
<tr>
<td></td>
<td>• Ulcerative colitis</td>
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<tr>
<td></td>
<td>• Rosacea</td>
</tr>
<tr>
<td></td>
<td>• Steven Johnson’s syndrome</td>
</tr>
<tr>
<td></td>
<td>• Sarcoidosis</td>
</tr>
<tr>
<td></td>
<td>• Behçet’s disease</td>
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<tr>
<td></td>
<td>• Psoriasis</td>
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<tr>
<td></td>
<td>• Malignancy</td>
</tr>
<tr>
<td></td>
<td>• Cryoglobulinemia</td>
</tr>
<tr>
<td></td>
<td>• Schönlein-Henoch purpura</td>
</tr>
<tr>
<td></td>
<td>• Serum sickness</td>
</tr>
<tr>
<td></td>
<td>• Pyoderma gangrenosum</td>
</tr>
<tr>
<td></td>
<td>• Erythema devatum diutinum</td>
</tr>
</tbody>
</table>

**HISTORY**

The ocular symptoms vary, but patients may complaint of a nonspecific foreign body sensation with or without pain, tearing, photophobia, and reduced visual acuity. The decrease in the visual acuity may be gradual or a sudden loss of vision may occur when PUK progresses. PUK is frequently a manifestation of an occult systemic disease. Thus, a thorough systemic history is very important and should include chief complaints, characteristics of present illness, past medical history, family history, and a meticulous systemic history. Sometimes an underlying systemic disease may be diagnosed by the presence of PUK.

PUK associated with RA, WG, PAN, and RP is associated with scleritis, and may also be associated with significant pain. PUK in patients with Mooren’s ulcer may also present with pain, although there is no scleral involvement.

The onset of ulceration should be noted as acute (onset of symptoms within 2 weeks of presentation), subacute (within 3 months) or chronic (> 3 months).

Past medical history should be sought especially in relation to a systemic disease such as RA, SLE, PAN, WG and RP. History should be taken for constitutional symptoms, such as chills, fever, decreased appetite, recent weight loss, and fatigue, any problems related to skin disorders, respiratory, cardiac, gastrointestinal and neurologic symptoms. This may elicit the presence of systemic disease, which may cause PUK.

**Examination**

Examination should include a complete systemic and a thorough ocular examination.

**SYSTEMIC**

Any lesion on the skin, face, trunk, joints, and extremities should be noted. Diagnosis of PUK in a patient of collagen vascular disease may require multiple evaluations by a rheumatologist or clinical immunologist as clinically detectable manifestations of these systemic diseases may evolve slowly.

**OCULAR**

A complete ocular examination should be performed with special emphasis on the conjunctiva, sclera, and cornea. Visual acuity, laterality, intraocular pressure and...
Peripheral Ulcerative Keratitis

detailed results of biomicroscopic slit-lamp examination should be recorded. Corneal sensations and lacrimal function by Schirmer test should also be recorded. Apart from this the details of anterior chamber (for the presence of any inflammation), vitreous, and fundus should also be noted.

Slit-lamp examination generally reveals a presence of a crescent-shaped lesion of the juxtalimbal corneal stroma associated with an epithelial defect, stromal yellow-white infiltrates composed of inflammatory cells, and varying degrees of corneal stromal thinning (minimal to full thickness) adjacent to the limbus. In severe cases, the peripheral cornea is progressively thinned out both circumferentially and centrally. The leading edge of the ulcer is infiltrated and untreated PUK will eventually progress towards the visual axis.

Corneal ulceration should be graded as follows:
< 25 percent depth of ulceration = 1, 25-50 percent = 2, 50-75 percent = 3 and 75-100 percent = 4.

Scleral involvement should be characterized as diffuse, nodular or necrotizing. PUK when accompanied by a necrotizing scleritis indicates the presence of an underlying systemic disease (Fig. 17.4).6

Investigations

The various investigations in a case of PUK includes systemic as well as ocular investigations. The tests for systemic evaluation should be done, wherever available. The various laboratory tests should focus on the suspected underlying systemic disease and include the following:

1. Complete blood cell count
2. Erythrocyte sedimentation rate
3. Serum creatinine, blood urea nitrogen
4. Rheumatoid factor (RF) in cases of RA (80% positive in RA)
5. Angiotensin-converting enzyme (ACE) which may be elevated in sarcoidosis
6. Antinuclear antibodies (ANA) which are positive in patients with SLE and RA
7. Antineutrophil cytoplasmic antibodies (ANCA); C-ANCA sensitivity of 96 percent for active generalized WG, 67 percent for active regional disease, and 32 percent for WG in full remission after initial regional symptoms9
8. Anti-type II antibodies (positive in RP)
9. Complement - C3 and C4, CH50; in patients with SLE
10. Hepatitis B surface antigen (HBsAg); present in 40 percent of patients with PAN.

Imaging Studies

Chest X-ray and sinus CT scan to rule out WG, sarcoidosis, and tuberculosis should be done and other radiographic studies of the affected joints should also be undertaken.

Microbiology Work Up

Routine scraping and culture of the ulcer are recommended in all cases (as described for microbial keratitis).10-12 PUK can be caused by microbial organisms such as bacteria, fungus or herpes. Hence in all cases corneal scraping should be done and smears should be prepared and cultures should be sent.

Biopsy

The conjunctival resection/biopsy is helpful in establishing an etiologic diagnosis in some cases and also helps in removing the limbal source of collagenases and other factors causing progressive ulceration. Biopsies are taken from the bulbar conjunctiva adjacent to the ulcerating cornea. In selected cases, episcleral, scleral and/or corneal tissue may also be excised and analyzed.

HISTOLOGIC FINDINGS

In cases of biopsied specimens any evidence of vasculitis, perivasculitis, granulomas, eosinophils, mast cells and...
Specific Types of Keratitis

Specific Types of Keratitis

neutrophil and lymphocyte infiltrate should be documented to corroborate the diagnosis of collagen vascular disease. However, vasculitis may be segmental and focal and hence a single negative biopsy does not rule it out.

In Mooren’s ulcer, corneal thickening occurs at the margin of the ulcer where inflammatory cells have invaded the anterior stromal layers. However, the inflammation is nonspecific, and no etiologic agent can be identified. Necrosis of the involved epithelium and stroma is seen.

**Treatment**

The treatment is aimed towards promoting epithelial wound repair and limiting ulceration and supporting the repair process.

**Medical Therapy**

The local treatment is aimed at preventing or reducing the epithelial defect, while systemic treatment is given to treat the underlying disease. One of the aims of treatment is epithelization of the ulcer, which will halt the progression of the corneal ulceration.

**Topical Therapy**

In cases where a microbial organism is suspected to cause PUK, antimicrobial therapy is started depending on the organism. However, in cases where microbial cause is ruled out, topical corticosteroids are the mainstay for treatment of PUK. Topical prednisolone acetate 1% eye drops four to six times a day are given which are tapered after the inflammation settles down.

Topical 1 percent medroxyprogesterone (which inhibits collagenase synthesis) is given in qid doses. Alternatively, topical 20 percent N-acetylcysteine (a competitive inhibitor of collagenase) may also be given in similar doses. Copious lubricants and ointments (preferably preservative free) in aiding re-epithelialization. Prophylactic topical antibiotics such as chloramphenicol 0.3 percent may be given in qid doses.

**Systemic Therapy**

**Antibiotics**

Systemic collagenase inhibitors such as tetracycline 250-mg tab qid or doxycycline 100 mg tab bid for the initial one month followed by tapering dose may help in slowing the progression of the pathology.

**Immunosuppressive Therapy**

It is generally seen that in cases of PUK associated with systemic diseases, recurrences following symptomatic treatment are common.

The systemic immunosuppression therapy is indicated in cases of PUK in the following situations:

1. PUK unresponsive to aggressive conventional medical and surgical therapy
2. Bilateral and/or progressive Mooren’s ulcer
3. PUK associated with potentially lethal systemic vasculitic syndromes, such as PAN, RA, SLE, RP, WG, PSS, Sjögren’s syndrome, allergic angiitis of Churg-Strauss, and giant cell arteritis
4. PUK associated with necrotizing scleritis with vasculitis based on histopathologic analysis.

**Corticosteroids**

High dose oral prednisone is started, as the chemotherapeutic agents begin to act optimally only after 4-6 weeks. Prednisone is given 1-1.5 mg/kg/d initially (not to exceed 60-80 mg/d) (Table 17.2). The dose is adjusted on the basis of the clinical response and adverse effects.

**Cyclophosphamide**

Cyclophosphamide is the drug of choice for almost all PUK associated with connective tissue disorders. Cyclophosphamide is used in the oral dose of 1 to 2 mg/kg/day or as pulsed intravenous therapy every 3 to 4 weeks under rheumatologist or internal medicine guidance (Table 17.2). The dose is adjusted to maintain the total blood count between 3000 and 4000 per microliter for optimal efficacy and safety.

**Methotrexate**

Methotrexate is preferred as the initial drug by some ophthalmologists over cyclophosphamide, as it is less toxic. Oral methotrexate 10 to 25 mg/week is given for the treatment of scleritis and PUK, which is unresponsive to corticosteroids.

**Cyclosporine**

In cases of recalcitrant disease, oral cyclosporin A is used in the dose of 2.5 to 5.0 mg/kg/day with frequent monitoring of blood pressure and renal functions (Table 17.2). The doses are adjusted so that the trough levels of whole blood cyclosporin are in the range of 150-200 ng/ml.
TABLE 17.2
Drugs given in a case of PUK

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adult dose</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>1 mg/kg/d PO initially; not to exceed 60-80 mg/d</td>
<td>Provides prompt suppression of inflammatory and immunologic reaction</td>
</tr>
</tbody>
</table>

**Immunosuppressive agents**—Inhibit key factors that mediate immune response

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adult dose</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate (Folex PFS, Rheumatrex)</td>
<td>7.5-12.5 mg/wk PO/IM/SC single dose initially; not to exceed 40 mg/wk</td>
<td>Hypersensitivity, hepatic insufficiency, immunodeficiency syndromes, pre-existing blood dyscrasias, renal insufficiency</td>
</tr>
<tr>
<td>Azathioprine (Imuran)</td>
<td>1-3 mg/kg/d PO initially</td>
<td>Hypersensitivity reactions</td>
</tr>
</tbody>
</table>

Precautions
- Monitor complete blood counts monthly and liver and renal function 1 to 3 monthly. Fatal reactions reported when administered concurrently with NSAIDs
- Increases risk of neoplasia,
  - May cause liver and kidney toxicity
  - Hematologic toxicities may occur
- Check liver function tests, kidney function tests and complete blood counts level prior to therapy
- Monitor liver, renal, and hematologic function

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adult dose</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin A (Sandimmune, Neoral, SangCyA)</td>
<td>2.5-5 mg/kg/d PO divided bid initially; not to exceed 10 mg/kg/d</td>
<td>Documented hypersensitivity; uncontrolled hypertension or malignancies.</td>
</tr>
<tr>
<td>Cyclophosphamide (Cytoxan)</td>
<td>Initial dose is 2 mg/kg/d PO; not to exceed 3 mg/kg/d</td>
<td>Hypersensitivity reactions; breastfeeding; immunosuppression; leukopenia or thrombocytopenia</td>
</tr>
</tbody>
</table>
| Precautions                         | Bone marrow depression may occur requiring regular blood count monitoring | Long-term oral therapy may lead to myelodysplasia
|                                     | Urinalysis required, malignancy, ovarian and testicular atrophy, l | Lymphopenia with opportunistic infections may occur

**TNF Alpha Blockers**

Recently, Infliximab (Remicade™, Centacor, Malvern, PA) a chimeric antibody against the proinflammatory cytokine tumor necrosis factor alpha (TNFα) has also been used (Table 17.2).\(^{15}\) It is effective for treatment of RA that was refractory to conventional disease-modifying drugs. Sterile corneal ulceration develops when there is an imbalance between MMPs and their physiological inhibitors, such as tissue inhibitors of matrix metalloproteinases (TIMPs), that may occur on the ocular surface of patients with RA. The target of infliximab, TNFα, is recognized to stimulate the production of the matrix metalloproteinases (MMPs) that are responsible for the dissolution of the corneal epithelial basement membrane and stroma in PUK.

Infliximab is given in three doses of 3 mg/kg IV initially and then at weeks 2 and 6 and then every 8 weeks for a period of 8 to 18 months.
Specific Types of Keratitis

Another systemically administered TNFα inhibitor, etanercept, which is a soluble TNF receptor, has also been observed to be effective for treatment of necrotizing scleritis and keratitis. Both of these anti-TNF agents can be used synergistically with methotrexate, which may enhance their effect. They have a much better safety profile than cyclophosphamide but do carry an increased risk of opportunistic infections.

When local or systemic infectious causes are suspected, therapy must be aimed at eliminating the infectious organism using the appropriate antibiotic medications based on clinical presentation or culture.

Referral to an appropriate specialist may be necessary. In patients with connective tissue diseases, co-management with a rheumatologist is necessary to address the systemic disease. Pulmonary, nephrology, cardiac, hematology, and infectious disease consultations may be sought depending on the patient’s symptoms and laboratory findings. Regular consultation with an oncologist may be necessary for those patients who are receiving chemotherapy.

SURGICAL TREATMENT

Tissue Adhesives

Tissue adhesives, such as cyanoacrylate glue, are recommended for use in impending perforation and perforation size smaller than 1-2 mm. Adhesive application follows keratectomy and conjunctival resection to remove sources of collagenase, cytokines, and inflammatory cells from the ulcerated cornea, temporarily preventing further stromal loss. Application of a bandage contact lens prevents discomfort and dislodging of the adhesive.

Tectonic procedures, including lamellar keratoplasty, penetrating keratoplasty, and corneoscleral patch grafts, are performed as needed to maintain the integrity of the globe when corneoscleral perforation is imminent or has occurred. A single stage or a multi-stage procedure may be undertaken for optimal visual rehabilitation.

FOLLOW-UP

Continued, possibly lifelong, follow-up care is necessary even after complete resolution since relapses may occur. Furthermore, many patients may require prolonged systemic steroid, non-steroidal anti-inflammatory, and/or chemotherapeutic medications for the systemic disease despite a quiet eye.

COMPLICATIONS

Ocular complications include corneal scarring and neovascularization with irregular astigmatism, corneal thinning and perforation, loss of vision, and even blindness.

PROGNOSIS

In patients with collagen vascular diseases, PUK with necrotizing scleritis is associated with poor life expectancy because of the presence of subclinical systemic vasculitis.

Specific Diseases

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease, which produces the arthritis of the hands, wrists, knees and feet. It is diagnosed on the basis of the diagnostic criteria followed by the American College of Rheumatology.

Keratoconjunctivitis sicca (KCS) is the most common ocular manifestation of the disease and is due to meibomian gland dysfunction. PUK is a common occurrence in RA and associated scleritis is a distinguishing feature. Marked peripheral thinning or marginal furrows rapidly progressive in nature can occur. No (Fig. 17.5) associated vascularization or epithelial defect is seen.

The patient’s clinical profile and positive serologic studies help in establishing the diagnosis. The 5 years mortality rate for untreated RA with either PUK or

Figure 17.5: Rheumatoid melt
scleritis is approximately 50 percent. Appropriate systemic therapy in addition to topical treatment helps in effective management of the disease.

**WEGENER’S GRANULOMATOSIS**

This is a rare multi-system granulomatous necrotizing vasculitis characterized by respiratory and renal involvement. Ocular involvement occurs in up to 50 to 60 percent. The associated conjunctivitis and scleritis may progress to PUK or PUK may be present as an isolated finding. The sclera is usually involved in these cases and this differentiates it from Mooren’s ulcer in which sclera is generally not involved.

A laboratory test, which helps in the diagnosis of Wegener’s granulomatosis, is the serum anti-neutrophil cytoplasmic antibody (ANCA) test. ANCA titers correlate with the severity and extent of the disease and tend to decrease in remission of the disease. Two patterns of staining are associated with this test—the C-ANCA (cytoplasmic anti-neutrophil cytoplasmic antibody) and the P-ANCA (perinuclear anti-neutrophil cytoplasmic antibody). The C-ANCA test has 99 percent specificity and 96 percent sensitivity. This test also helps to follow the clinical response to therapy and chances of recurrence of PUK are more if these values have not normalized, despite apparent clinical remission when therapy has been tapered or discontinued.

**POLYARTERITIS NODOSA**

PAN is a rare multi-system disease with necrotizing vasculitis of the small and medium sized arteries. Histopathological identification of the vascular changes is diagnostic of PAN. The various ophthalmic manifestations include choroidal vasculitis (most common ophthalmic manifestation), PUK, conjunctival lesions, scleritis, choroiditis, central retinal artery occlusion are the various ophthalmic manifestations. The clinical characteristics of PUK in this disease are similar to those of Mooren’s ulcer. Hepatitis B surface antigen is positive in about 50 percent of patients with PAN. Systemic immunosuppressive therapy is the key to retard the progression of PUK.

**OTHER COLLAGEN VASCULAR DISEASES (CVD)**

PUK is rarely seen in other CVD such as systemic sclerosis, SLE and RP. The clinical profile and the laboratory tests help in confirming the diagnosis. The detection of antibodies to double stranded (dsDNA) is specific for SLE. No laboratory test is specific for relapsing polychondritis. Dry eye is seen in about 70 percent of systemic sclerosis patients. PUK may occur unrelated to the KCS.

**STAPHYLOCCAL MARGINAL KERATITIS**

Staphylococcal marginal keratitis occurs in a patient with chronic blepharitis. This presents as a peripheral infiltrate with breakdown of the overlying epithelium. Immune complex mediated reactions against the microbial antigens have been attributed to cause the ulceration.

**TERRIEN’S MARGINAL DEGENERATION (TMD)**

TMD is usually non-inflammatory and does show features of corneal ulceration. It is more common in males and is asymmetrical. TMD begins superiorly as fine punctate stromal opacities and a clear zone exists between the limbus and the infiltrate. Superficial vascularization is also present. However, the epithelium overlying is intact. The peripheral thinned zone is determined by a white lipid line at its central edge. Slowly progressive thinning spreads circumferentially and causes irregular astigmatism. An oblique pseudopterygium is associated in about 20 percent of cases and perforation may occur due to trivial trauma. Recurrent inflammation, scleritis/episcleritis is rarely seen.
Specific Types of Keratitis

SENILE FURROW DEGENERATION (SFD)
SFD is thinning of the interval between the limbus and the arcus senilis and occurs in the elderly.

ROSACEA KERATITIS
Ocular rosacea is a relatively common disorder. The diagnosis largely rests on its association with the cutaneous disease characterized by persistent erythema, telangiectasia, papules and pustules and hypertrophic sebaceous glands of the face and neck. Ocular involvement is seen in up to 58 percent of cases and ranges from chronic blepharoconjunctivitis to neovascularization and thinning. PUK is rarely associated with ocular rosacea.

The lesion begins as vascularized marginal subepithelial infiltrates, which are initially small and round. Untreated cases progress to ulcers spreading towards the center of the cornea (Fig. 17.8). Oral tetracycline (250 mg 4 times/day) or doxycycline (100 mg bd) is effective along with topical corticosteroids which is given for 4 to 6 weeks duration.

MOOREN’S ULCER

Definition
Mooren’s is a chronic. It is characterized by the presence of an overhanging edge overlying central and leading edge that starts in the periphery and may progress centrally or circumferentially to involve the entire cornea (Fig. 17.9).

Males have a greater predilection for this disorder (1.6 times).16

Types of Mooren’s Ulcer

Mooren’s ulcer has been classified into 2 groups according to the age of onset and the clinical characteristics.17 Type I is benign and usually unilateral with mild to moderate symptoms. It occurs in older people (over 35 years) and usually responds well to medical and surgical treatment (Fig. 17.9). Type II is malignant and occurs in younger patients (< 35 years) and is more likely to be bilateral (in 75% cases) with relatively more pain and poor response to therapy (Fig. 17.10).

Pathogenesis
Autoimmunity is suspected to be involved in the pathogenesis of Mooren’s ulcer based on evidence of circulating antibodies18,19 to the corneal stroma and specific cell mediated immune reaction toward a partially purified corneal antigen (Co-Ag). Reports have described the presence of inflammatory cells, immunoglobulin and increased expression of HLA class II molecules in the cornea and conjunctiva, adjacent to the ulcers.20

The triggering factor instigating this autoimmune response is not clearly known. Privileged corneal antigens may become the target of the patient’s immune system by local exposure of these antigens due to corneal trauma, surgery or infection.21,22 It has been proposed that organisms such as a helminth23 stimulate the
Peripheral Ulcerative Keratitis

production of antibodies that cross-react with corneal antigens and cause ulcer. Mooren’s ulcer has occurred following corneal trauma and eye surgery.\textsuperscript{22}

**Histopathology**

Pathological examination of the specimens of involved corneal tissue have shown presence of plasma cells, neutrophils, mast cells and eosinophils in the involved regions.\textsuperscript{24} There was destruction of the collagen matrix. Epithelium and Bowman’s layer were absent. Midstroma showed hyperactivity of fibroblasts with disorganization of the collagen lamellae. The deep stroma was intact but contained heavy macrophage infiltration. Heavy neutrophil infiltration and dissolution of the superficial stroma were present at the leading edge of the ulcer. Adjacent conjunctiva shows epithelial hyperplasia and a subconjunctival lymphocytic and plasma cell infiltration.

**Clinical Features**

Patients with Mooren’s ulcer will complain of redness of the eye, tearing and photophobia, but pain is typically the outstanding feature. The pain is excruciating and may seem well out of proportion the corneal inflammation. Decreased visual acuity may be secondary to associated iritis irregular astigmatism due to the peripheral corneal thinning.

The disease may begin as several patchy peripheral stromal infiltrates that then coalesce, commonly in the region of the palpebral fissure. Generally there is involvement upto the limbus. The ulcerative process spreads circumferentially and then centrally to involve the entire cornea eventually. The anterior 1/3 to 1/2 of the stroma is involved characteristically with a steep overlying central and leading edge. Healing and vascularization occurs slowly over 4-18 months. Parts of the ulcer may be quiescent while the remaining may be active (Fig. 17.11). The end stage is a typical scarred, vascularized thinned cornea with the patient experiencing sudden relief from the excruciating pain.

Adjacent conjunctiva may be inflamed and viritis is sometimes associated with Mooren’s ulcer. Hypopyon is rare unless secondary infection is present. Sometimes there may be associated glaucoma and cataract. Perforation is rare, though it can occur, especially following trivial trauma due to the presence of weakened cornea (Fig. 17.12).

**Diagnosis**

Investigations include laboratory investigations to rule out several systemic diseases leading to PUK. These include immunology and dermatology workup, X-ray chest and sinus X-ray, Mantoux, hemogram, liver and renal function tests, rheumatoid factor, antinuclear antibody (ANA), antineutrophil cytoplasmic antibody (ANCA), complement fixation, angiotensin converting enzyme, VDRL, hepatitis B, hepatitis C and HIV antigen detection, serum protein electrophoresis and stool examination. Scrapings should be done to rule out infectious pathology per se or secondary infection due to Mooren’s ulcer.
Specific Types of Keratitis

Treatment

The management of Mooren’s ulcer depends on the presentation of the disease and can be done both by medical or surgical means depending on the severity of the disease.

MEDICAL MANAGEMENT

The mainstay for medical management of Mooren’s ulcer is topical corticosteroids. These are given in 4 to 6 hourly dosage initially. However, if healing is not satisfactory the dose may be increased to 1 hourly frequency. Generally 1% prednisolone acetate eyedrops are used.

Gradual tapering of topical steroids over a period is done during the healing phase of the disease. It is imperative to maintain the patient on topical dilute steroids over a prolonged period. Use of oral pulse steroids (60-100 mg of oral prednisolone) has also been advocated where topical therapy is ineffective for over 10 days or in cases of deep ulcers. Topical tetracycline, medroxy progesterone 1 percent may be used because of their acute collagenolytic properties. A therapeutic bandage contact lens may be used in conjunctiva along with topical therapy. Prophylactic topical chloromphenicol eyedrops are also given in qid doses.

Some people also give topical cyclosporin A 2% eyedrops in qid doses. However, this is not found to be very effective.

In malignant cases systemic corticosteroid should be added. Systemic prednisolone is given in the dose of 1.5 mg/per/kg body weight.

SURGICAL MANAGEMENT

The surgical management of Mooren’s ulcer include limbal conjunctivectomy, keratoepithelioplasty and keratoplasty.

Limbal Conjunctivectomy/Resection

 Conjunctival resection may be done if the ulcer progresses despite steroid therapy. The excision and recession of the adjacent limbal conjunctiva aids in the removal of proteolytic conjunctival enzymes or the inflammatory cells that produce antibodies against the cornea. A wide conjunctival resection to bare the sclera, extending at least 2 clock hours on either side of the ulcer and 4 mm posteriorly has been recommended.

Keratoepithelioplasty

In keratoepithelioplasty several fresh donor corneal lenticules with intact epithelium are placed near the distal side of the ulcerated area and securely sutured on the bare sclera with 10.0 nylon interrupted sutures.
The lenticules form a biological barrier between the host cornea and the conjunctiva which is the source of immunological mediators. This procedure can be combined with a corneoscleral lamellar graft.

**Superficial Keratectomy**

Superficial lamellar keratectomy along with conjunctival resection including the resection of the overhanging lip of the ulcerating cornea and application of tissue adhesive with bandage soft contact lens application or amniotic membrane has been described.

**Keratoplasty**

In advanced cases surgery may be done in two stages, that is initial lamellar tectonic grafting followed by central penetrating keratoplasty. Lamellar keratoplasty removes antigenic targets of the cornea, prevents immunological reactions, reconstructs the anatomical structure, prevents perforation and improves vision (Figs 17.13A and B).

**References**

Surgical Management

SECTION 5
**Introduction**

Intracameral route of drug administration is gradually emerging as a new modality for the management of deep keratitis particularly those due to fungal etiology. In severe cases of fungal keratitis, hyphae may penetrate an intact Descemet’s membrane and colonize in the anterior chamber.\(^1\) The hypopyon in these cases of deep keratomycosis usually contains fungal elements (unlike bacterial corneal ulcers with perforation where the hypopyon is sterile).\(^2\) Fungal hypopyon is particularly difficult to treat because corneal penetration of most topically applied anti-fungal drugs is poor and improves only marginally even with repeated scraping of the corneal epithelium.\(^3\)

Thus in these cases of deep keratitis with retrocorneal involvement or anterior chamber involvement, adequate drug levels may not be achieved at the site of the infection.\(^4\) Both, the antibiotics as well as anti-fungal agents have been used intracameral in various studies. However, with the advent of the newer generation antibiotics such as fluoroquinolones, which have excellent ocular penetration, the intracameral mode of anti-bacterials is not indicated. Presently, the use of intracameral antimicrobials as a modality for the management of infectious keratitis is limited to treat fungal keratitis.

**Indications of Intracameral Antimicrobials**

Only amphotericin B has been used to treat fungal keratitis caused by yeasts and natamycin-resistant filamentous fungi, notably *Aspergillus*\(^5\)\(^-\)\(^8\). Intracameral amphotericin B is given in addition to the topical and systemic antimicrobial therapy. In experimental studies it has been demonstrated that anterior chamber injection of as much as 50 micrograms of amphotericin B does not cause any lenticular or corneal toxicity.\(^9\)

**TECHNIQUE**

**Preparation of Intracameral Amphotericin B Injection**

Commercial preparations of amphotericin B (50 mg powder, Fungizone, Sarabhai Chemicals, Vadodara, India) for intravenous administration is diluted for intracameral injection. To achieve the desired concentration, the drug is diluted aseptically in preservative-free sterile water for injection.

**Dosage**

Amphotericin B is constituted in 5 percent dextrose. Ten milliliters of dextrose is added to the vial, giving a mixture containing 5.0 mg/mL; 1.0 mL of this is added to 4.0 mL of dextrose to further dilute it to 1.0 mg/mL. Of this concentration, 1.0 mL is then diluted with 9.0 mL of dextrose, thus making a concentration of 100 μg/mL; 0.1 mL of this contains 10 μg. Similarly, 0.75 and 1.25 mL of this diluted drug contains 7.5 and 12.5 μg, respectively.

**Surgical Technique**

Anterior chamber paracentesis for instillation of the intracameral drugs should be performed under the operating microscope in the operating room under sterile conditions.

Topical anesthesia is used and 0.5 percent proparacaine is instilled at 5 minutes intervals three times prior to injection. The anterior chamber entry is made from the superior or the temporal limbus as these are most accessible areas, and preferably from a site where clear
cornea is seen which aids in visualization of the track of the needle. A 22 gauge needle is usually used. Viscoelastic such as hydroxypropyl methylcellulose may be used for viscoexpression of the anterior chamber exudates. If the exudate cannot be removed, the anterior chamber may be entered with no. 11 surgical blade and the exudate removed with forceps or a Simcoe cannula may be used to aspirate the exudate. Care should be taken not to damage the corneal endothelium, especially when endothelial plaque is being aspirated. Further, sometimes it may become necessary to leave a part of the exudate, especially if the anterior capsule is involved, as this may injure the lens and cause cataract.

Smears should be prepared for KOH wet mount preparation, Gram’s and Giemsa stains and culture media such as blood agar, chocolate agar, non-nutrient

![Figure 18.1: Case of fungal keratitis (Aspergillus)](image)

![Figure 18.2: Fungal keratitis treated with Intracameral amphotericin B (same case as Figure 18.1)](image)

**TABLE 18.1**

<table>
<thead>
<tr>
<th>Author, year</th>
<th>No.</th>
<th>Age/sex</th>
<th>Initial VA</th>
<th>Ulcer size (mm)</th>
<th>Dose of amphotericin B (µg)</th>
<th>No. of injections</th>
<th>Final VA</th>
<th>Organism</th>
<th>Outcome</th>
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<tr>
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<td>1</td>
<td>50/F</td>
<td>CFFC</td>
<td>3 x 4</td>
<td>5</td>
<td>4</td>
<td>CF 1 m</td>
<td>Fungus</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55/M</td>
<td>PL, PR</td>
<td>5 x 6</td>
<td>5</td>
<td>13</td>
<td>CF 2 m</td>
<td>Fusarium</td>
<td>Healed</td>
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<tr>
<td></td>
<td>3</td>
<td>53/M</td>
<td>CF 1/2 m</td>
<td>2.4 x 2.2</td>
<td>5</td>
<td>3</td>
<td>20/200</td>
<td>No growth</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td>4</td>
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<td>6 x 8</td>
<td>5</td>
<td>4</td>
<td>-</td>
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<td>20/200</td>
<td>Aspergillus flavus</td>
<td>Healed</td>
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<td></td>
<td>6</td>
<td>42/M</td>
<td>HMCF</td>
<td>2.5 x 3.5</td>
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<td>2</td>
<td>-</td>
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<tr>
<td></td>
<td>7</td>
<td>28/M</td>
<td>HMCF</td>
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<td>1</td>
<td>20/80</td>
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<tr>
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<td>CF 3 m</td>
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<td>20/70</td>
<td>Aspergillus</td>
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<tr>
<td></td>
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<td>28/M</td>
<td>20/400</td>
<td>Abscess with endothelial exudate</td>
<td>5</td>
<td>2</td>
<td>20/50</td>
<td>Fungus</td>
<td>Ulcer healed. Cataract surgery done</td>
</tr>
</tbody>
</table>
Intracameral Antibiotics

agar, and thioglycollate broth should be inoculated. Intracameral injection of amphotericin B 7.5 mg in 0.1 ml is prepared as described previously and injected. The wound is then closed with a 10-0 monofilament nylon suture.

Repeat Injections

Repeat injections are given based on the clinical response. In cases of non-responding cases, a repeat injection may be considered after a time interval of 1 to 5 days.

Generally the response to intracameral amphotericin B is monitored by the following parameters: A clinical response is present when the size of the epithelial defect, ulcer infiltrates and hypopyon decreases. The corneal infiltrates become rounded, there is visible shrinking of the endothelial plaque and pigmentation on the surface of the endothelial plaque (Figs 18.1 and 18.2). Although a maximum of 13 injections have been reported, we prefer not to give more than 4 injections. These injections are given from the site of the first injection.

RESULTS

In the various studies in literature as well as in our experience intracameral amphotericin B is safe and efficacious in treating deep keratomycosis and non-responsive mycotic keratitis (Table 18.1). The patients may complain of pain immediately after the injection and there may be transient uveitis and an exudative membrane during the first 24 hours. The time from the first injection to the complete resolution of the endothelial plaque ranges from 13 to 52 days. The various organisms, which have been isolated from the anterior chamber tap, include Cladosporium species, Acanthamoeba, Aspergillus species and Cryptococcus species.

Summary

Presently, the intracameral injection of antimicrobials is used only to treat some cases of deep mycotic keratitis.

References

Introduction

Tissue adhesives such as cyanoacrylate glue has been used in various ophthalmic conditions. These include repair of frank or impending corneal perforations, closure of leaking filtering blebs, occlusion of lacrimal puncta, skin closure in oculoplastics (e.g. skin grafts) and fixing of hard/rigid contact lenses in severe ocular surface disease (e.g. chemical burns).

Chemical Composition and Characteristics

There are two types of tissue adhesives—the synthetic (in the form of cyanoacrylate derivatives) and the biologic (in the form of fibrin glue). Of the various cyanoacrylate derivatives, butyl monomers with optimum adhesive strength and rate of polymerization have been reported to be effective in the treatment of corneal perforations up to 3 mm in diameter. Cyanoacrylates are esters (alkyl side chains) of cyanoacrylic acid and the polymerization (hardening) of the glue is due to the active double bond with oxygen.

The histotoxicity of the cyanoacrylates depends on the length of the alkyl side chains and the vascularity of the tissues on which it is being applied. It is greater when the alkyl side chains are short and when the glue is being used in the well-vascularized soft tissues.

The accumulation of the degradation products in the tissues leads to significant tissue toxicity characterized by both acute and chronic inflammation. The currently available glues (e.g. N-butyl-2-cyanoacrylate) have longer alkyl chains and hence degrade slowly thereby limiting the accumulation of byproducts to amounts that can be effectively eliminated by tissues. The polymerization (hardening) of cyanoacrylates occur through contact with water or a weak base (such as cell membranes/tissue pH) and the hydroxylation occurs through the exclusion of oxygen from the substances being bonded.

Fibrin glue is a highly concentrated fibrinogen aprotinin solution, which also contains Factor XIII. A solution of thrombin and calcium chloride are applied to the ulcerated area, where the mixture coagulates. The presence of Factor XIII causes the fibrin to crosslink which gives the coagulum additional resilience.

Biological Effects

It was shown that direct early application of cyanoacrylate adhesive to the ulcer bed and adjacent basement membrane along with a bandage contact lens was effective in preventing progression of the corneal stromal melting. The corneal epithelium and its interaction with subjacent keratocytes stimulate the infiltration of the corneal stroma by polymorphonuclear neutrophils and subsequent release of collagenolytic enzymes in corneal ulcerations. Cyanoacrylate glue prevents re-epithelialization into the zone of damaged stroma and hence inhibits polymorphonuclear leukocytes, which are the source of collagenolytic and proteolytic enzymes. Interruption of the melting process is most successful when applied early in the course before overwhelming numbers of polymorphonuclear neutrophils have accumulated.

Cyanoacrylate glue also possesses antibacterial properties both in vitro and in vivo. It has significant bacteriostatic activity against gram-positive organisms (Staphylococcus aureus, Streptococcus pneumoniae, and group A streptococci).

Application of the Glue

PATIENT SELECTION

The indications for using cyanoacrylate glue include temporary repair procedure of small corneal perforations (less than 2 mm diameter), descemetoceles and corneal melts (Fig. 19.1).
METHOD OF APPLICATION

The cyanoacrylate glue may be applied with various devices, which include tuberculin syringe, applicators and aerosol application.9-12

Materials Required

Histoacryl glue
1 mL syringe and 26 gauge needle
Weckcel sponges

Direct Application method:

- The glue is loaded in a 1ml tuberculin syringe with a fresh 26 G needle.
- Topical anesthesia with 0.5 percent proparacaine eye drops is instilled.
- The lid speculum is inserted gently as the eye is perforated and soft.
- The surface is dried completely.
- The adjacent epithelium is scraped.
- If the anterior chamber is flat and the defect is large (>2 mm), the anterior chamber may be reformed with air or viscoelastic to enable easier application of the glue.
- The syringe is then gently screwed in small turns (for controlled release) to express the glue onto the 26 G needle tip. Alternatively the bead of the glue may be dripped on to a fresh 26 G needle tip for better results.
- The region to be treated is touched with the tip of the needle containing the glue drop.
- After waiting for 2 minutes, a bandage contact lens is applied.

MODIFICATION OF THE TECHNIQUE

The adherence of the glue is limited by the size of the defect and the condition of the surrounding tissue to which the glue is bonding (Fig 19.2). Reapplication may be required in case of continued corneal stroma melting or persistent wound leak. Various modifications have been made in the application of the glue. These include the following:

Overlapped Applications

Larger defects can be treated with multiple applications overlapped if necessary.

Using a Suture

A running 10/0 nylon suture is used to create a reticulum over the defect on which the glue is applied in case of larger defects (> 3 mm).13 This is followed by the application of the bandage contact lens.

Viscoelastics

The sodium hyaluronate can be used as a visco-maintainer/retainer prior to application of the corneal glue in situations where anterior chamber is compromised and/or intraocular contents are tending to prolapse through the perforation site.9 The viscoelastic is useful as it prevents the intraocular structures from adhering to the glue.

Corneal Patch

A dermatological punch is used to cut three or four circular patches of the non-stick portion of opsite plastic
Surgical Management

or the steridrape. The opsite is picked with a stick, which is dipped in a K-Y jelly and the cyanoacrylate glue is applied over the patch with a 26 gauge needle which is mounted on a tuberculin syringe and this patch is then applied at the site of the perforation (Figs 19.3A and B).

This procedure is effective in healing of the small corneal perforations. In larger perforations such as in cases of sterile melts this procedure helps in delaying surgery which can be performed as an elective procedure at a later date under more optimal conditions of controlled inflammation.

POSTOPERATIVE MANAGEMENT

Topical prophylactic antibiotic drops should be used at 2 hourly intervals for the first three days followed by four times daily. Additionally, lubricants should also be given. One should monitor the degree and time to epithelialization, vascularization, and corneal thickness. Any evidence of extrusion of the glue or infection, especially at 3 to 5 weeks should also be be monitored.

ADVERSE EFFECTS

Application of cyanoacrylate glue to the conjunctiva, sclera or skin causes greater tissue reaction than to the corneal epithelium and stroma. The tissue histotoxicity is generally less with the newer cyanoacrylates. The side effects of the glue application include the scarring of the conjunctiva and giant papillary conjunctivitis.14,15 Inadvertent instillation of cyanoacrylate glue into the anterior chamber can result in polymerization of the corneal endothelium and iridocorneal and iridolenticular adhesions.16 Close monitoring for infection/corneal infiltrate is essential when the glue has been present for more than 6 weeks especially with the therapeutic contact lenses. Rare report of retinal toxicity, cataracts and granulomatous keratitis are also there.15

References


Introduction

Over the years, conjunctival flaps have been used to treat recalcitrant corneal ulcers. However, with the advent of better lubrication systems, hydrophilic bandage contact lens, tissue adhesives, more potent antimicrobial agents, collagen shields and amniotic membrane transplantation, the routine use of conjunctival flaps is steadily diminishing.

Presently, conjunctival flaps are performed for only a selected group of patients of corneal ulcers.

Indications

A conjunctival flap is principally used to treat recalcitrant sterile corneal ulcers. Conjunctival flaps provide a smooth ocular surface and also help in tectonic support and nutrition to a chronic, non-healing corneal ulcer.

The major indications of conjunctival flaps are:
1. Neurotrophic corneal ulcers
2. Neuroparalytic keratitis
3. Exposure keratitis
4. Peripheral ulcerative keratitis

Conjunctival flaps may be rarely used in non-responding fungal and bacterial keratitis with damage to epithelial basement membrane damage, which hinders the surface healing. However, most corneal specialists do not like to perform conjunctival flaps due to the following reasons:
1. Conjunctival flaps obscure the view of the ulcerated area hindering the monitoring of the progression of corneal ulcer.
2. Penetration of the antimicrobial agents may be suboptimal.

Contraindications

The contraindications of conjunctival flaps include the following:
1. Corneal perforation
2. Infectious corneal ulcers.

Types of Conjunctival Flaps

The conjunctival flaps may be of two types depending on type of conjunctival closure – partial or total. The partial conjunctival flaps may be advancement flaps, bipedicle flaps or single-pedicle flaps.

Partial Conjunctival Flaps

Advancement Flaps

An advancement conjunctival flap is be used to cover a peripheral limbal or paralimbal corneal lesion such as following a limbal dermoid excision. A limbal incision with relaxing incisions is made and the flap is advanced over the peripheral lesion and sutured onto the conjunctiva (Fig. 20.1). Peripheral patch grafts and scleroconical grafts may also be covered by these advancement flaps. However, such flaps usually retract and gape over a period of time.

Single Pedicle Flaps (Racquet Flaps)

The flaps were advocated for peripheral and paracentral lesions, which are not large enough to require a complete flap. Subconjunctival lidocaine with 1:100,000 epinephrine may be used to balloon the area of the conjunctiva to be undermined. The adjacent pedicle of conjunctiva is fashioned and is placed on the peripheral corneal lesion and sutured. Although technically more difficult than a simple advancement flap, it has the advantage that the retraction of the flap does not occur.

Bipedicle Flaps (Bucket Handle Flaps)

These are used for small paracentral or limbal corneal lesions that do not require the coverage of the whole cornea. The width of the flap should be 1.3 to 1.5 times
Conjunctival Flaps

the width of the lesion. The flap is fashioned with two pedicles from the limbal conjunctiva spanning the width of the lesion and placed on the lesion and fixed with sutures.

**Total Conjunctival Flap (Gunderson Flap)**

This is performed for large lesions requiring coverage of the total cornea. The superior bulbar conjunctiva is ballooned with injection of 2 ml of 2 percent lignocaine with 1 in 100,000 dilution of adrenaline into the subconjunctival space anterior to the Tenon’s capsule. This aids in easy dissection. The bulbar conjunctiva starting from the superior fornix is dissected free from the Tenon’s capsule up to the 10 and 2 O’clock positions. All tension on the flap is relieved by dissection.

After debridement of the corneal epithelium, the pedicle is placed over the cornea. In cases of irregular corneal surface a superficial keratectomy may be required. The cornea is then irrigated meticulously with balanced salt solution and care is taken to maintain the correct orientation of the flap. The flap is then positioned on the cornea and anchored to the limbus or sclera with interrupted 7-0 silk or other non-absorbable material sutures (Fig. 20.2). The superior sclera is left bare which re-epithelialises rapidly. An inferior flap can be similarly fashioned if required.

**Postoperative Therapy**

Postoperatively, a broad-spectrum fluoroquinolone such as 0.3 percent ciprofloxacin or ofloxacin is given four times a day for one week along with a cycloplegic agent. The suture removal is done at one month.

**Complications**

The complications may occur intraoperatively or postoperatively.

**Intraoperative**

Intraoperatively, button-holing of the conjunctiva may occur which should be avoided by careful and meticulous dissection of the conjunctiva. In case a button-hole occurs, it should be sutured with a non-absorbable suture.

**Postoperative**

Postoperatively, retention of the conjunctival cyst may occur due to in curling of the conjunctival edges. However, these do not cause too much of a problem and may be left alone.

Further, occurrence of the herpetic infection in the conjunctival flap has also been reported.

**Summary**

Conjunctival flaps have limited usefulness in the modern day era. They may be used in cases of indolent painful corneal ulcers. Successful keratoplasty is possible after performance of these flaps.
5 Surgical Management

References

Introduction

Therapeutic keratoplasty is usually performed to manage a corneal perforation in a case of corneal ulceration. Sometimes it is used as a modality of treatment to debulk the cornea of active infection that is not getting controlled with maximal medical therapy. Despite recent advances in medical management of infectious keratitis a sub-group of microbes may not respond to antimicrobial therapy so that a therapeutic keratoplasty is indicated when the disease progresses in spite of maximum medical therapy and/or the integrity of the globe is compromised. This procedure helps by the following methods:
1. Excision of the infectious or inflammatory process by debulking or removing the infectious inoculum so that the organisms in the cornea decreases to a level at which exogenous infective process terminates and the host defense becomes active.
2. It maintains the integrity of the globe integrity.
3. It may provide visual rehabilitation in some cases.
4. It may also help in diagnosis of the infective pathology.

Therapeutic keratoplasty is of great relevance especially in developing countries where cases of non-healing microbial keratitis and perforated corneal ulcers are frequent and therapeutic keratoplasty is frequently performed.

Indications

The primary indication of a therapeutic graft is a perforated corneal ulcer that is not amenable to corneal gluing. It is also performed for uncontrolled microbial infections particularly the fungal keratitis to debulk the cornea of infectious tissue.

BACTERIAL KERATITIS

Penetrating keratoplasty is required less often for the treatment of active bacterial keratitis owing to the availability of specific antibacterial drugs. Indications for keratoplasty in bacterial keratitis include progressive ulceration despite maximum antibacterial medication (Fig. 21.1), extensive corneal involvement and formation of descemetocele, or perforation (Fig. 21.2).

Figure 21.1: Non-healing corneal ulcer on maximal anti-bacterial therapy

Figure 21.2: Perforated corneal ulcer due to Pseudomonas
Surgical Management

The percentage of cases requiring a therapeutic keratoplasty in cases of bacterial corneal ulcer varies from 3-6 percent. The most common organism isolated in these cases includes *Staphylococcus* species. The other organisms, which have also been isolated in various studies, include *Proteus* species, *Streptococci pneumoniae*, *Moraxella* species, and *Salmonella* species. Other indications of therapeutic keratoplasty are the conditions that are resistant to conventional medical treatment like infectious crystalline keratopathy and *Mycobacterial* keratitis.

**Fungal Keratitis**

Therapeutic keratoplasty is more often required as compared to cases bacterial keratitis in the management of refractory fungal corneal ulcers. The incidence of cases requiring a therapeutic keratoplasty in fungal keratitis varies from 18-29 percent. The availability of effective and newer anti- *Acanthamoeba* medications early and moderately advanced cases may be successfully treated with medical treatment alone. In the management of advanced cases, which are unresponsive to medical therapy a therapeutic corneal transplantation alone or in combination with cryotherapy of the host cornea has been recommended. Although some surgeons recommend early keratoplasty in cases of *Acanthamoeba* keratitis to remove the bulk of infiltration and to reduce the risk of scleral extension, presently a therapeutic penetrating keratoplasty in *Acanthamoeba* keratitis is performed for advanced cases that are unresponsive to medical management. A review of 38 case reports of *Acanthamoeba* keratitis in various studies of different authors showed that 29 patients required a transplant surgery.

**Herpes Keratitis**

A therapeutic keratoplasty may need to be performed for necrotizing herpetic keratitis leading to extensive corneal melting and perforation. Therapeutic keratoplasty is also performed in patients who develop corneal perforations secondary to persistent epithelial defect with little or no stromal inflammation.

**Other Indications**

Apart from infectious keratitis, therapeutic keratoplasty is also undertaken in large corneal perforations secondary to keratoconjunctivitis sicca as in Sjögren’s syndrome, *Staphylococci pneumoniae*, *Moraxella* species, and *Salmonella* species. Other indications of therapeutic keratoplasty are the conditions that are resistant to conventional medical treatment like infectious crystalline keratopathy and *Mycobacterial* keratitis. Other indications of therapeutic keratoplasty are the conditions that are resistant to conventional medical treatment like infectious crystalline keratopathy and *Mycobacterial* keratitis.

**Timing of Surgery**

Timing of the surgery also relates to the success of the therapeutic graft. A few reports suggest that a delay in the surgery improves the surgical outcome. Nobe et al obtained a graft clarity rate of 17 percent with emergency surgery (within 24 hours), 57 percent with intermediate surgery (within 2-6 days) and 31 percent with a delayed surgery (1 week-2 months). Failure rate was highest if the surgery was an emergent one. In a study by Foster et al a graft clarity rate of 85 percent was achieved in eyes that were initially managed with lamellar keratoplasty or glue and bandage contact lens and a delayed penetrating keratoplasty compared to 17 percent in eyes treated with a early penetrating graft for a perforation. Polack et al reported clear grafts in all the
eyes with inactive herpes compared to a rate of 45 percent in grafts with active disease.\(^3\)

**Preoperative Evaluation**

**HISTORY TAKING**

History taking should include a detailed assessment of onset of symptoms, duration of symptoms, prior medical therapy and/or any surgical intervention, information about the type of organism isolated (if the records are available) and the response to medical therapy.

**OCULAR EXAMINATION**

Prior to the surgery a detailed ocular examination is mandatory. Initial assessment includes determination of best-corrected visual acuity and slit-lamp evaluation to determine the depth and extent of the corneal involvement and size of the infiltrates particularly in relation to the limbus.\(^2\)\(^-\)\(^4\) This facilitates planning of surgical details such as size of trephine that has to be used and its optimal placement in the recipient’s bed.

The presence of iris prolapse is diagnostic of a corneal perforation. Topical fluorescein and a positive Seidel’s test should be done to evaluate the size of a small perforation (Figs 21.5A to C). The amount of anterior segment inflammation is assessed carefully and the eye is examined for other associated conditions like dry eye, exposure, secondary glaucoma and endophthalmitis.\(^3\)\(^0\)\(^,\)\(^4\)\(^0\)

**INVESTIGATIONS**

A and B scan ultrasonography is mandatory when the integrity of globe is not compromised by a large perforation, to assess the posterior segment involvement and to rule out endophthalmitis especially when retina is not visualized adequately.\(^2\)

**PREOPERATIVE TREATMENT**

Preoperatively, the patients may be given antibiotic agents, antiglaucoma medications and some surgeons even recommend the use of corticosteroids.

**Anti-microbial Therapy**

Both systemic and topical anti-microbial treatment is recommended preoperatively in all the patients.\(^2\) The treatment should be directed against the offending agent. If preoperatively an etiologic diagnosis has not been established, broad-spectrum antibiotic or a combination therapy should be given.\(^2\) Donnenfeld et al recommend use of topical ofloxacin at hourly frequency in all cases undergoing therapeutic keratoplasty regardless of the cause of infection to prevent bacterial superinfection. They also recommend use of Ofloxacin 400 mg every 12 hours before admission and
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intravenous vancomycin and tobramycin after hospitalization.³

Steroids
Most of the authors²,³,⁷,¹⁰,¹¹ avoid use of topical steroids as it worsens the infectious process, though Killingsworth⁴ and O’Day et al⁴¹ recommend use of topical and systemic steroids even in cases of fungal keratitis.

Hypotensive Agents
Many authors recommend preoperative use of intravenous mannitol to lower the intraocular pressure.²⁻³ We routinely use intravenous mannitol in all cases preoperatively. This decreases the problems related to positive vitreous pressure.

Our Regime
We in our practice start the patients on systemic Ciprofloxacin 500 mg twice a day and Acetazolamide 250 mg three times a day especially in cases of perforated corneal ulcers. Topical treatment includes use of cefazoline sodium (5%) and tobramycin sulphate (1.3%) at hourly frequency. Alternatively Gatifloxacin (0.3%) eye drops may also be combined with cefazolin sodium (5%) at an hourly frequency. Homatropine (2%) eye drops are also given thrice a day. We avoid the preoperative use of topical and systemic steroids.

Surgical Technique

ANESTHESIA
Therapeutic keratoplasty should always be performed under general anesthesia by experienced corneal surgeons as local anesthesia carries the danger of compromising the integrity of the perforated globe.⁴ The anesthetist should be informed about the caution with the use of depolarizing agents to avoid sudden intraocular pressure rise in an open globe.³⁴⁻²⁻³

DONOR TISSUE CONSIDERATIONS
A good quality donor cornea should be used as a healthy tissue with intact epithelium minimizes the risk of reinfection in the graft and the presence of clear graft helps in monitoring the anterior chamber reaction during the postoperative period.² If fresh donor is not available one can also use cryo-preserved and glycerin-preserved corneas and even sclera has been suggested.⁴⁴⁻⁴⁶

HOST TISSUE PREPARATION
Exposure
A self-retaining lid speculum or lid sutures are helpful in preventing the pressure on the globe.³ Killingsworth⁴ suggested that in most of the cases the scleral fixation rings should be avoided, as in a hypotonous eye the suturing is difficult, whereas others advocate³⁴⁻⁷ use of a Flieringa ring whenever possible to provide scleral support. We do not use scleral fixation rings owing to their difficult suturing in eye with very low tension.

Conjunctival peritomies should be performed in cases which require a large or an eccentric graft to avoid passing sutures through the conjunctiva.²⁻⁴ Hemostasis should be achieved in all cases of conjunctival peritomies with a wet field cautery.²

Recipient Bed Trephination
The size of the recipient bed should be determined by placing the appropriate trephine over the cornea and creating an indent in the epithelium keeping in mind the size of graft and the centration. During trephination of the recipient bed, care should be taken to avoid pressure on the globe which could lead to extrusion of the ocular contents. Trephination is difficult in eyes with large perforations, which have zero IOP. The recipient cornea is incised using either a hand held or a vacuum trephine. A comparative study done by John D Ng et al on eyes with both central and peripheral large corneal perforations suggested that the Hessberg-Barron and Hanna suction trephines are easier to use as compared to Franceschetti type free-blade trephine.⁴⁷

The suction trephines have lesser incidence of anterior chamber collapse and iris prolapse compared to the free-blade trephines. In suction trephines peripheral suction provides the support and counter-traction into the advancing blade whereas the free-blade relies solely on the inherent structural stability of cornea which is already weak in cases of perforations. The authors found that the Hanna trephine was technically more easy to use than the Hessberg- Barron trephines.⁴⁷ It has more peripheral ring of suction that causes less central corneal distortion and holds the cornea in a more natural configuration.⁴⁷

We use disposable handheld trephine in all cases due to easy availability. Some surgeons advocate initial
tectonic support with cyanoacrylate glue or a patch graft in cases of eyes with perforation.\textsuperscript{2} They also recommend freehand dissection of the host bed after marking with a trephine, in cases of large perforation or with entire cornea involvement.\textsuperscript{2}

**Size of the Recipient Bed**

The smallest recipient opening is created that encompasses the ulcerated or infiltrated corneal tissue completely. The basic aim is to have a central trephination of a standard size of 7 to 8 mm as far as possible. However, the diameter of host trephination usually depends on location and size of perforation and surrounding infected area. If the size of perforation is small and peripheral, a small patch graft may be suitable. If the perforation is very large reaching up to limbus or involving sclera beyond the limbus a 12 to 13 mm graft may be required. Care should be taken to remove the entire diseased area even if the size of the graft required becomes larger.\textsuperscript{2,4} Whenever possible, the host trephination should include at least 1 mm of healthy corneal tissue.\textsuperscript{3}

**Anterior Chamber Reconstruction**

Purulent and fibrinous material should be irrigated from the anterior chamber; the membranes over the iris should be dissected gently by the irrigating cannula and should be removed with the forceps and iris lesions should be excised.\textsuperscript{48} Every effort should be made to arrest the bleeding from the iris surface. One or two iridectomies is recommended by most of the authors.\textsuperscript{2-4} Donnenfeld et al\textsuperscript{2} and Rao et al\textsuperscript{3} suggest use of intracameral antibiotics or antifungals whenever required. Intracameral vancomycin 0.1 mg/ml and intracameral Amphotericin B 7.5 micrograms/ml wash may be given.

**Lens Surgery**

In most cases one should try to preserve the lens as it forms an effective barrier against the spread of infection into the vitreous.\textsuperscript{2-4} Donnenfeld et al recommends use of 2 percent pilocarpine 1 hour preoperatively in all phakics and pseudophakics in an attempt to constrict the pupil and prevent spontaneous expulsion of the lens.\textsuperscript{3}

**Anterior Vitrectomy**

If associated endophthalmitis is found at the time of surgical procedure, a combination of therapeutic keratoplasty, crystalline or intraocular lens removal, total open sky vitrectomy, and injection of appropriate intraocular antibiotic at the end of the surgery is advocated.\textsuperscript{2,4} Even in cases of suspected endophthalmitis, if the organism is known, appropriate antibiotic should be injected intravitreally and vitreous tap should be taken for culture. If the organism is not known intravitreal injection of vancomycin (1 mg in 0.1 ml) and ceftizidime (2.5 mg in 0.1 ml) should be given.\textsuperscript{3} Open sky vitrectomy is performed in all aphakics and in cases complicated by inadvertent crystalline lens extrusion and vitreous loss.\textsuperscript{2} The anterior chamber is reformed gently with a viscoelastic agent and the peripheral anterior synechiae are released to open up the anterior chamber angle.\textsuperscript{2,4} Over more monofilament sutures may be applied to reconstruct the pupil.

**DONOR TISSUE PREPARATION**

In a case of therapeutic keratoplasty, the donor button is trephined always after the recipient’s trephination, as necrosis around the host cut may require additional trimming and may alter the size of the graft.\textsuperscript{2} Careful decision regarding the graft size should be made. With a very small graft and an eccentric graft the sutures may come into the visual axis resulting in a visual compromise. However, a large graft may be associated with the danger of graft rejection, post-keratoplasty glaucoma and subsequent graft failure.\textsuperscript{49,50} Hence, careful assessment of the size and centration is mandatory. The clarity of the graft depends on the size of the graft. Larger grafts give a worse prognosis because of more chances of immunologic graft reactions with vascularization, development of posterior synechiae and secondary glaucoma. Du and coworkers reported a clarity rate of 89 percent with a graft size of 7 mm or less compared to 21 percent where grafts were 8 mm or larger.\textsuperscript{51}

Donor button is punched from the endothelial side, and is usually 0.5-1.0 mm larger than the host bed.\textsuperscript{2,4} We take 1 mm oversized grafts in cases of perforated corneal ulcers as this helps in better maintaining of the anterior chamber postoperatively.\textsuperscript{48}

**GRAFT HOST APPOSITION**

Donor button is sutured in place with 10-0 monofilament nylon interrupted. Use of interrupted sutures is preferred by us and also by most of the surgeons.\textsuperscript{2,4} The suture depth should be 75 percent of the host corneal thickness and not full thickness as this increases the risk for
conduit of the infectious organism from cornea to the anterior chamber. Longer sutures bites on the host side with moderate tension are preferred to avoid cheese wiring of the suture through a potentially necrotic bed. All suture knots should be trimmed and buried on the host side (Figs 21.6A and B). Meticulous wound closure is recommended even if it requires greater number of sutures. Care should be taken to avoid any pressure over the globe throughout the surgery.

Microbiological and Pathological Examination

In all cases, the corneal specimen is divided into two pieces and sent for microbial and histopathological investigations. Material should be inoculated on blood agar, chocolate agar, Sabouraud’s agar without cyclohexamide and blood agar coated with \textit{E. coli}, for plating. The corneal specimen should also be sent for histopathological evaluation for identification of the offending organisms.

Postoperative Management

The postoperative therapy consists of the use of antimicrobial agents and cycloplegics.

ANTIMICROBIAL THERAPY

There is a general consensus to give antimicrobial postoperatively following therapeutic keratoplasty. The duration of therapy depends on the severity of infection and the causative organism and should be given until the corneal epithelium has healed. Fungal, Acanthamoeba and viral keratitis may require antimicrobial treatment for several months post-operatively.

In cases of bacterial keratitis we give topical fluoroquinolones such as 0.3 percent ciprofloxacin or 0.3 percent ofloxacin eye drops are given 2 to 4 hourly initially along with systemic antibiotics.

In cases of fungal keratitis, in addition to the above regime, 5 percent natamycin eye drops five times a day are also added.

In cases of herpetic keratitis, in addition to the antibiotic, topical acyclovir 3 percent ointment is added 5 times a day in cases of keratitis subsequent to herpes simplex and systemic acyclovir is added in case of keratitis subsequent to herpes zoster.

In cases of acanthamoeba keratitis, in addition to the antibiotics, polyhexamethyl biguanide or brolene eye drops are given.

STEROIDS

The use of topical steroids following a therapeutic keratoplasty for infectious keratitis is controversial. Killingsworth et al and O’Day et al suggested that as the infection is cured by surgery in virtually all the patients, aggressive use of both topical and systemic steroids can be considered postoperatively in all cases to decrease the postinflammatory sequel and improve the visual outcome.

As most of the bacterial corneal infections are responsive to antibiotics therefore concomitant use of corticosteroids may be justified in an inflamed eye.
We give topical steroids in cases of bacterial keratitis in reduced frequency (8 hourly doses). However, in cases of recurrence of infection, and in cases of active fungal or Acanthamoeba infection after therapeutic keratoplasty steroids should be avoided.\(^4\) In therapeutic keratoplasty for herpetic keratitis, topical corticosteroids may be given without significant risk, as long as the patient is managed with concomitant topical or oral antiviral therapy.\(^41\)

**Complications**

The complications seen after therapeutic keratoplasty include severe uveal inflammation, and reinfection of the graft along with other usual complications seen after a standard full thickness corneal transplantation surgery.

**HEMORRHAGE/HYPHEMA**

Postoperative hyphema results from inflamed iris and if associated with raised intraocular tension requires evacuation\(^2\) (Figs 21.7A to C).

Mild to moderate anterior uveitis is encountered in almost all cases (40-100\%)\(^6,7,51-53\) and can be controlled by judicious use of topical corticosteroids and cycloplegics (Figs 21.8A and B). O’Day et al advocates use of steroids even in fungal corneal ulcers.\(^6,7,41\) Severe iritis may lead to formation of pupillary membrane in 3 to 9 percent cases.\(^7,52\)

**SECONDARY GLAUCOMA**

Early postoperative increase in IOP(14-20\%) occurs secondary to anterior chamber reaction and may require topical and systemic anti-glaucoma treatment along with anti-inflammatory therapy.\(^2\)

Late onset secondary glaucoma results from extensive peripheral synechiae and may lead to graft failure. The incidence of secondary glaucoma has been reported to vary from 3 percent in keratoplasty for Pseudomonal keratitis\(^6\) to 50 percent after keratoplasty in fungal corneal ulcer.\(^6,52,53\) Our study showed a 19.46 percent incidence of glaucoma following therapeutic keratoplasty.
Figures 21.9A and B: (A) Recurrence of infection in a therapeutic graft (B) Uncontrolled infection in the same case

RECURRENT OF INFECTION

Recurrence of infection is the most undesirable complication of therapeutic keratoplasty.

A therapeutic keratoplasty in active herpetic keratitis has been associated with highest reinfection rate (7-75%) compared to the other infectious keratitis. Polack et al report a recurrence in 15(75%) of the 20 patients who underwent keratoplasty for acute viral keratitis, although 9 (60%) grafts survived the early recurrence. Recurrence rate in keratomycosis varies from 7.3-10 percent. Acanthamoeba keratitis has been associated with a recurrence rate of 40-50 percent. In our study the graft reinfection rate was seen in 9 eyes (7.96%) (Figs 21.9A and B).

It should be considered as an emergency and should be treated intensively with topical antimicrobial therapy and a repeat surgery may be undertaken, if required.

GRAFT FAILURE

Graft clarity is the most important factor in deciding the functional outcome of the procedure. Graft failure following therapeutic keratoplasty occurs in 4 to 52 percent. The status of the graft depends as a number of factors. These are offending organism, timing of surgery, donor tissue quality and the graft size.

OTHER COMPLICATIONS

Other complications following therapeutic keratoplasty include, graft ectasia (7% in early postoperative period and 16% in late postoperative period), chronic irritable eye (7%) and phthisis bulbi (1%).

SUCCESS OF A THERAPEUTIC GRAFT

The final visual outcome after therapeutic keratoplasty depends on the virulence of the causative organism, its susceptibility to treatment, the severity of inflammation, the timing of the surgery, the type of donor material used, the size of the graft, underlying ocular surface disorder and the postoperative management of various complications.

Acanthamoeba keratitis and keratomycosis have a worse prognosis as compared to bacterial keratitis. Therapeutic grafts, which remain clear in cases of bacterial keratitis, range from 70 to 75 percent in comparison to fungal keratitis where it varies from 20 to 60 percent and Acanthamoeba keratitis which has a graft clarity rate up to 50 percent.

Summary

Therapeutic penetrating keratoplasty has a definitive role in the management of infectious keratitis especially when integrity of the globe is jeopardized. Results of the procedure can be improved by concomitant use of specific antimicrobial therapy both preoperatively and postoperatively. It is mandatory to encompass and remove the entire infected area even if a large sized graft is required. Further long-term studies are required to evaluate the outcome of therapeutic keratoplasty with
a good quality donor, which can replace requirement of the second stage optical keratoplasty.

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Surgical Management

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Introduction

Phototherapeutic keratectomy has been done by various investigators in cases of healing keratitis. It may be difficult to perform surgery in these inflamed and infected eyes.

Phototherapeutic keratectomy has been evaluated earlier as a surgical tool to treat superficial corneal pathologies such as recurrent epithelial erosions, corneal dystrophies degenerations and corneal scars involving the anterior one-thirds of the stroma.

The ability of 193 nm excimer laser to treat microbial keratitis was demonstrated initially by Serdaveric et al in rabbit corneas after scratch inoculation with Candida albicans.\(^1\) It has been shown to be effective in early localized Fusarium, Mycobacterium and Pseudomonas keratitis in animal models.\(^1\)\(^-\)\(^3\)

MECHANISM OF ACTION

Due to the ultraviolet radiation, tissue sterilization occurs and this effect is further enhanced due to ablation or elimination of organisms and the surrounding necrotic tissue.\(^2\) It also provides debulking effect and improves drug penetration.

CASE SELECTION

Phototherapeutic keratectomy can be undertaken in cases of superficial keratomycosis which do not respond to standard antimycotic therapy. There are only a few topical and systemic antifungal drugs available for the management of keratomycosis which often penetrate the ocular barriers poorly. It has been reported that 20 to 50 percent cases of keratomycosis require surgical intervention.\(^4\)

Phototherapeutic keratectomy may also be done in cases of superficial opacities due to herpetic keratitis. It may also be done in cases of long standing metaherpetic ulcers which do not epithelialize on conventional treatment. Excimer laser can also be used in selected patients having opacities and irregularities due to herpes simplex keratitis.\(^5\)

CASE WORK UP

Slit-lamp biomicroscopy is mandatory in cases where phototherapeutic keratectomy is planned. The optical pachymeter should be used to measure the total corneal thickness and the apparent involvement of the suppurrative stroma.\(^2\) In cases where there is substantial corneal thinning that is more than 50 percent of the cornea, phototherapeutic keratectomy should not be undertaken. It should also be noted that following phototherapeutic keratectomy the eyes become hyperopic and hence the refractive status and vision of the fellow eye should also be considered.

LASER CHARACTERISTICS

The ability of 193 nm excimer laser to eradicate a variety of microorganisms as colonies in agar plates has been reported.\(^9\) Ultrastructural findings in cases treated with the 193 nm wavelength laser consist of fine basophilic stippling at the epithelial- stromal interface in the treated area. The underlying stroma which has not been ablated is undamaged on light microscopic examination.\(^1\)

Complications

Few complications following phototherapeutic keratectomy for treatment of infectious keratitis have been reported.\(^5\)\(^,\)\(^7\) Since the corneal thickness in cases of keratitis is irregular and the ablation rate of the suppurating corneal tissue is not predictable, a conservative approach towards excimer laser ablation should be adopted. Usually one-third to one-half of the corneal thickness is considered as a safe margin for ablation.
Surgical Management

Pachymetry may be unreliable in these cases and it may be difficult to reveal the thinnest point on the cornea.

Astigmatism may occur which occurs due to the difference in the ablation rates of infected and normal tissues, size and depth of the lesion and involvement of the optical axis. A focal lesion in the paracentral area will be thinner as compared to the adjacent normal tissue and hence induce astigmatism. The initial zone for phototherapeutic keratectomy should be broad enough to cover the entire optical zone.

Delayed epithelialization may occur in some cases. Corneal haze is more common especially in fungal keratitis as topical corticosteroids are often not used in these cases.

Conclusion

Topical medical therapy should be continued after phototherapeutic keratectomy in all cases. Great caution should be used in selecting excimer treatment candidates because of high-risk of corneal perforation in advanced infections. Recurrences have been reported especially in cases of herpes simplex keratitis.

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