# Histopathology and molecular diagnosis of corneal infections

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## Abstract

Infectious keratitis is an important cause of visual loss worldwide. Clinical diagnosis in the past was often supported only by microbiology and pathology to a lesser extent. Recent advances in the histological and molecular diagnosis of corneal infections have resulted in rapid and accurate diagnosis of the infectious agent. This review will provide an overview of the various corneal infections, with emphasis on histopathologic and molecular diagnosis. This is more so in cases where microbiology, the gold standard for corneal infections, comes out as negative. Thus a cumulative input from clinical, microbiology, histopathology and molecular methods of diagnosis not only helps in treating the patients but also contributes to better understanding of the disease process and paves the way to evaluate the emerging modalities of treatment like disease modifying medications, biomaterials and surgical techniques.

**Keywords** corneal histopathology; corneal infiltrate; microbial keratitis; molecular diagnosis; polymerase chain reaction

## Introduction

Microbial keratitis is an important cause of ocular morbidity worldwide, the outcome of which depends on early diagnosis, prompt and effective treatment and various host and agent factors.[1] Some of the common causes of corneal infections include bacterial, fungal, viral, and protozoan, the diagnosis of which is made on clinical examination aided by microbiological demonstration in smears or cultures from corneal tissues. In advanced cases however, therapeutic or diagnostic indications necessitate procedures like corneal biopsy, penetrating keratoplasty or even evisceration of the eye, which thus provides an opportunity to the pathologist to aid in early diagnosis and thus initiation of early treatment. Histological evaluation and molecular methods not only aid in diagnosis but also improve our understanding of the disease pathogenesis of this unique avascular, unarmed, and transparent tissue. This chapter provides a brief outline of clinical features and treatment of specific infections with emphasis on histopathology and molecular methods of diagnosis.

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## Histopathology of corneal infections: general considerations

Unlike other tissues of the body where orientation is not a big concern, corneal tissues require edge embedding to retain proper orientation of corneal layers. Histological features of infectious keratitis reflect the features seen on slit lamp examination or by confocal examination of the eye. The severity of the disease process, rate of progression, response to treatment, complications differ in different types of corneal infections. However, in general, uncontrolled infections usually go through the phase of epithelial ulceration, destruction of Bowman's layer, stromal infiltration by polymorphonuclear (PMN) and lymphomononuclear cells, necrosis of stroma, breakdown of Descemets membrane, and ultimately perforation of cornea. Suppurative infections like bacterial and fungal lead to infiltrates in anterior 2/3 of stroma and abscess formation. Chronic infections show epithelial regeneration, vascularization, edema, giant cell reaction, myofibroblastic transformation and stromal remodelling (scarring) and round cell infiltration. Table 1 provides a guideline on evaluation of corneal layers in infectious keratitis. In addition to routine haematoxylin and eosin (H & E) and periodic acid Schiff's stain (PAS); appropriate special stains are useful in identifying the organisms.

## Histopathology of specific infections

### Bacterial keratitis

**Clinical presentation and treatment:** predisposing factors include ocular trauma or injury, contaminated water and eye drops, contact lens use, post-surgery, epithelial defects, ocular surface disease and systemic conditions.[1] Only a few microorganisms, notably *Neisseria gononorrhoea* can penetrate intact epithelium. Etiologic agents include gram-positive bacteria like *Staphylococcus* and *Streptococcus*. *Pseudomonas* and other Enterobacteriaceae are the primary gram-negative pathogens involved in microbial keratitis. Gram-negative infection shows relatively a rapid pace of inflammation, often leading to severe corneal abscess and perforation with hypopyon. For infection with gram-positive organisms, fortified cefazolin is the treatment of choice. For other bacteria, and gram-negative bacteria, ciprofloxacin or fourth generation fluoroquinolones are agents of choice. If diagnosed early, the infection can be limited and topical corticosteroids can be commenced once the culture-sensitivity is available. Rapid thinning often necessitates tissue adhesive application. In general, therapeutic keratoplasty is required far less often than in fungal keratitis and has a fair prognosis.

**Histopathology:** bacterial infections result in epithelial ulceration, with destruction of Bowman's layer and anterior stroma with severe and diffuse infiltration by PMNs. The stromal

**Summary of histologic features of corneal infections**

| Anatomic layer | Histologic features to be observed | Remarks |
|---|---|---|
| General features | Thickness, thinning, necrosis, perforation, separation, exudates, pigmentation. | |
| Epithelium | Intactness, edema, ulceration, hyperplasia, downgrowths inflammatory infiltrates, giant cell reaction, cytoplasmic inclusions regularity/breaks of basement membrane, pannus formation (inflammatory or degenerative). | Periodic acid Schiff's (PAS) stain is complementary to Haematoxylin and Eosin. |
| Bowman's layer | Thickness, breaks, absence, calcification, degenerative changes, any deposits. | Special stains as and when required. |
| Stroma | Thinning, edema, vascularization, inflammation and density and type of inflammatory cells (neutrophils, lymphocytes, plasma cells, giant cells), location of cells (anterior/mid/posterior stroma), perforation, cellularity, changes in keratocytes (myofibroblastic transformation, loss of keratocytes) orientation of collagen fibres, fibrosis, scarring, abnormal deposits, any infectious agent and its load and location. | Special stains as and when required for fungus, bacteria, *Acanthamoeba* and microsporidia. |
| Descemet's membrane (DM) & endothelium | Thin, fragmented or intact.<br>Giant cell reaction around DM, granulomatous inflammation around fragmented ends.<br>Presence of microorganisms (like fungus), presence and adequacy of endothelial cells, morphology of endothelial cells, retrocorneal membrane and exudates adherent to DM, anterior chamber exudates. | PAS stain, GMS. |
| Others | Adherent uveal tissue, AC exudates. | |

**Table 1**

thinning and destruction is contributed by collagenolytic enzymes released by the PMNs and bacterial endotoxins which leave behind nuclear debris. If left unattended, it results in perforation with herniation of iris into the site formatting a pseudocornea. Cyanoacrylate glue, if applied, could be seen as refractile wavy unstained glue on the surface of cornea with scalloping margins, appreciated better with the lowered condenser of the microscope. Bacteria on histologic sections are appreciated when present in colonies and with the use of Gram's stain. Unusual patterns of bacterial keratitis can be seen in infectious crystalline keratitis, commonly seen in corneal grafts or with the use of steroids. Bacterial colonies develop a biofilm thus appearing as discrete and viable colonies with fine, needle like extensions within the corneal stroma, (resembling crystals) with minimal stromal inflammation. The most common organism implicated is alpha-haemolytic streptococcus.[1]

### Fungal keratitis

**Clinical presentation and treatment:** fungal keratitis is a major blinding disease and accounts for upto 44% of central corneal ulcers in South India.[2] Organisms commonly implicated are *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp. and *Candida* sp. Dematiaceous fungi include *Curvularia* sp.[2] Approximately half do not respond to medical therapy and need surgical intervention. Predisposing factors include: trauma (vegetative matter), contact lenses, post-surgery (after PK), use of corticosteroids and chronic keratitis like viral keratitis. Large dry raised infiltrate with feathery or hyphate margins is pathognomic of this infection.[2] Diagnosis is based on identification of **s**eptate hyaline filaments on Gram's stain fluorescent filaments on potassium hydroxide calcofluor white preparation (KOH—CW) and fungal growth on most media. Confocal scan is a promising modality for the in-vivo diagnosis of fungal keratitis, especially in deep seated keratitis. Intensive medical therapy with topical natamycin or amphotericin B is successful in anterior stromal infection. Advanced disease necessitates therapeutic penetrating keratoplasty with recent trends favoring lamellar keratoplasty which involves removal of only the anterior corneal lamella leaving behind the posterior stroma and Descemet's. While this is an advantage for many corneal diseases, presence of fungus in posterior stroma, Descemet's and anterior chamber hampers its use in severe fungal keratitis.

**Histopathology:** corneal epithelium is usually ulcerated, accompanied by edema, severe inflammation and stromal thinning. Density and extent of inflammation, necrosis depends on the mode of injury, duration of insult, treatment received and the local and systemic condition of the host. We observed that in the early stages the inflammation is focal, patchy and mostly involves the anterior 2/3rds of the stroma; with satellite lesions or abscesses in the surrounding stroma. The posterior stroma when affected may show loss of stromal keratocytes due to apoptosis (Figure 1b). Later these abscesses become confluent, extend to deep stroma, and lead to total destruction of stromal architecture with necrosis and perforation. Predominantly deep-seated lesions along with anterior chamber exudates and hypopyon, with relative sparing of